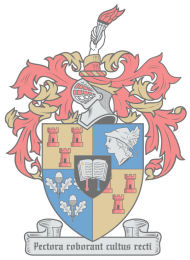
Carcinus maenas in South Africa: status, impacts and management

Clova Airth Mabin



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Dissertation presented for the degree of Doctor of Philosophy (Zoology)

Department of Botany and Zoology

Stellenbosch University

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Co-supervisors:	Prof John Wilson
	Prof Jaco Le Roux

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

I would like to declare that Dr Henning Winker at the Department of Agriculture, Forestry and Fisheries developed the code for the Bayesian depletion model used in Chapter 3. This allowed me to use the data collected during management of *Carcinus* to estimate the population's response to the intervention.

Throughout the thesis, the term *Carcinus* is used when referring to South African populations of crabs from this genus. This is a result of the detection of hybrids between *C. maenas* and *C. aestuarii* in Chapter 5, and the recognition that the results obtained in this thesis can thus not be ascribed to a particular *Carcinus* species.

During the management programme, I played the role of lead scientist, co-ordinating the research and contributing to decisions regarding the management plan.

During the course of my doctoral studies, I also completed the field work for and published the following paper: Mabin CA, Wilson JR, Robinson TB (2015) The Chilean black urchin, *Tetrapygus niger* (Molina, 1782) in South Africa: gone but not forgotten. BioInvasions Records 4: 261–264. Originally this work was to be incorporated into my thesis, with an envisaged management programme to control the urchin. However, when the surveys were conducted, no urchins were detected and the species was declared no longer present in South Africa.

Clova Mabin

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ABSTRACT

The European shore crab, *Carcinus maenas,* has established invasive populations on four continents, with its congener, the Mediterranean shore crab, *C. aestuarii,* invading another. *Carcinus maenas,* in particular, has caused ecological and economic impacts in several regions which led to targeted management. This crab was first detected in Table Bay harbour, Cape Town, South Africa in 1983, with little research undertaken in this region until the current study. Research conducted two decades ago suggested the presence of interspecific *Carcinus* hybrids in South Africa and accordingly, unless specified otherwise, the crab will be referred to as *Carcinus* only from hereon. This thesis firstly conducted a detailed assessment of the current population status in South Africa, followed by the undertaking of an intensive management programme. This intervention offered a rare opportunity to experimentally assess the trophic impacts of this crab in the field and apply genetic techniques to reconstruct its invasion history in the South African context.

To establish the current status of the species in South Africa, Chapter 2 assessed the distribution and abundance of *Carcinus*. This involved the first account of seasonal patterns in abundance and demographics for the crab. Six intertidal sites were visually surveyed during shore walks on a monthly basis for a year, with surveys extended quarterly at one site for another year. An additional four sites outside the known range of the species were surveyed quarterly, resulting in a total of 92 intertidal surveys through time over the ten sites. Subtidal surveys were conducted at three sites along the open coast. Baited traps were used to confirm the presence of the crab in two harbours (Hout Bay and Table Bay). Following this, Table Bay harbour was surveyed monthly for one year while targeting three life stages (postlarvae, juveniles and adults). The crab was only found at one intertidal site and no crabs were found during open coast subtidal surveys. Gravid females were recorded in all months except for February - May. Unexpectedly, no postlarvae were detected, suggesting that settlement collectors are ineffective in the South African context and should not be used for future monitoring. These results highlight a decline in open coast populations and substantial harbour populations. This suggests that management of harbour populations of *Carcinus* only, would be sufficient to remove the species from South Africa.

Due to the ecological impacts of this genus elsewhere, the *Carcinus* population in Hout Bay was targeted for management, with the aim of assessing the feasibility of removing the crab from the two invaded South African harbours. This first attempt at managing a marine invasive species in Africa is documented in Chapter 3. Prior to management, four potential control methods (baited traps, crab condos, diver collections and sediment dredging) were trialled to establish their efficacy and suitability for use on a large scale in the South African context. Baited traps were selected as the most appropriate and cost-effective method. Management was conducted from June 2014 to June 2015. During this period,

approximately 21,800 traps were deployed and a total of 18,450 person hours were spent in the field. This resulted in the removal of 36,244 crabs from the harbour, approximately six times the most recent population estimate. Despite this, extirpation was not achieved. The recovery of the population was then monitored for six months. A Bayesian depletion model was developed to simulate the response of the population under management scenarios of varying effort. This approach suggested that even if control effort had been doubled (i.e. to 8,000 hours per month) there would only have been an 86% probability of extirpation. If this effort were to be applied in both Hout Bay and Table Bay harbours, it would cost an estimated ZAR 60 million. This cost would need to be considered against the threat posed by the invasion and the likelihood of reintroduction.

Despite the impacts reported from invasive populations elsewhere in the world, the trophic impacts of *Carcinus* remain unknown in South Africa, therefore, this was addressed in Chapter 4. The management of *Carcinus* provided an opportunity to experimentally assess the impact of the crab by comparing community structure in Hout Bay and Table Bay using benthic cores and vertical fouling scrapes collected prior to (2014), during (2015) and after (2016) management. A total of 78 tethering experiments were conducted to identify potential predators of the crab in Hout Bay harbour and a large public aquarium setting (as a proxy for natural habitats in the absence of *Carcinus* in such systems), resulting in 128 hours of underwater camera footage. Benthic communities were similar between harbours prior to management, but contrary to expectations, the community remained similar through time in the managed harbour, while changing in the control harbour. In contrast, fouling communities differed between harbours at the onset and changed at both locations through time. Thus, the crab appears to have no detectable impact on benthic communities while its impact on fouling communities remains uncertain. While, no tethered crabs were attacked in Hout Bay, under aquarium conditions all crabs were preyed upon by native fish, suggesting that predator-driven biotic resistance might constrain the spread of *Carcinus* in natural habitats. Thus, despite notable impacts elsewhere, impacts of *Carcinus* were not detected in this study, highlighting that alien species impacts can vary among regions and context-dependent risk analyses are needed to inform evidence-based management.

In order to elucidate the historical and contemporary gene flow between selected international populations and South Africa, microsatellite DNA markers were used to assess the genetic diversity and structure of *Carcinus* populations across the native and invasive ranges in Chapter 5. To determine if the management action in Hout Bay had a detectable genetic impact on the demographics of the crab, Hout Bay and Table Bay were sampled prior to, during and after management. DNA was extracted from 898 samples, collected from eight international and the two South African populations (across three years) and amplified across eight microsatellite loci. Clustering analyses and approximate Bayesian computations (ABC) were performed to reconstruct the invasion history of the South African populations. South Africa had significantly higher genetic diversity than the other alien

Carcinus populations, suggesting a lack of a founder effect. In the most supported ABC invasion scenario, the South African populations established following admixture between native and invasive *C. maenas* populations approximately 150 years ago. Evidence of interspecific hybridization between *C. maenas* and *C. aestuarii* was detected, with the presence of several hybrid genotypes (5% of all individuals sampled). The harbours had a similar genetic diversity and no structure, suggesting migration between the populations or the same founding populations. There was no effect of management on genetic diversity, its structure, or effective population size of Hout Bay. Migration from Table Bay is, however, unlikely to be the dominant explanation of such observations. Rather, the most likely explanations are that a cohort of crabs produced prior to management was detected during sampling and reflected the gene frequencies of the previous generation or management interventions did not reduce crab populations below levels required to observe changes in genetic diversity. Given the probability of migration between the South African populations and the potential for re-introduction from international populations, the probability of successful management is considered low.

This thesis utilised approaches from several different disciplines, including ecology, population genetics and environmental management, to better understand *Carcinus* invasions in South Africa and to undertake the first management of a marine alien species on the continent. Despite the recent listing in national legislation that mandates the management of this crab, the results of this project suggest that (1) there is a lack of evidence of trophic impacts, (2) migration occurs between the harbours, (3) there is evidence for a complex introduction history to South Africa and appreciable levels hybridization between different crab species, and (4) there are administrative challenges to be addressed prior to management, including insufficient financing. It is thus recommended that a national eradication programme not currently be implemented. Importantly, while this project has provided responses to locally relevant questions, the scientific and administrative insights gained have broad applicability within the field of invasive alien species management. The scientific insights include the value of trialling methods used elsewhere, as well as the use of depletion models to estimate the effort required to increase the probability of management success. Often managers of invasive species rely on dated population estimates and impacts assessed elsewhere to inform management decisions. However, based on the outcomes of this study, management undertaken prior to the assessment of such factors can be unsuccessful and result in a sub-optimal use of limited resources. Overall, this work highlights the importance of context-specific field studies and molecular approaches to establish the need for and determine the feasibility of management.

CHAPTER 1. GENERAL INTRODUCTION

Marine invasions

Alien species are an important and increasing threat to biodiversity (Hulme 2009). The coastal marine environment is especially threatened by invasions, as marine alien species often go unnoticed until they are well established, sometimes with irreversible consequences (Bax et al. 2003, Coulatti et al. 2006, Molnar et al. 2008). Although lagging behind studies of biological invasions in terrestrial and freshwater ecosystems (Grosholz 2002), marine invasion research is becoming more extensive and accordingly, insights are being provided into the true status and impacts of marine invasive species around the world (Robinson et al. 2016). The movement of previously isolated species around the world's oceans has been on-going since the advent of shipping and counts of marine alien species are increasing with time (Ricciardi 2007, Hulme 2009). The principal vectors for the introduction of marine invasive species are ballast water discharge from commercial vessels and biofouling (Carlton 1999, Minchin et al. 2009). Ballast (water and solid) was historically considered the most important vector for marine invasions (Hewitt et al. 2004). However, since tributyltin (TBT) was banned in anti-fouling paints by the International Maritime Organisation (IMO 2001), biofouling has become an increasing threat (Lewis et al. 2004).

The detection and management of marine invasive species is challenging due to the high connectivity and few limitations on larval dispersion in the marine environment. Nonetheless, barriers to dispersal do exist, especially at more localised scales (Thresher & Kuris 2004, Cowen et al. 2006). Coastal bays in particular, can present a partial barrier to larval recruitment and dispersal (Byers & Pringle 2006) and in many regions, harbours are often located in such bays. With the confluence of numerous vectors, the availability of artificial habitat and potential barriers to local dispersal outside of harbours, these areas often form invasion foci (Peters et al. 2014).

Marine invasive species management

Successful eradications of marine invasive species are rare, with a few examples from Australia, New Zealand and the United States of America (Culver & Kuris 2000, Bax et al. 2002, Wotton et al. 2004, Miller et al. 2004, Anderson 2005, Hopkins et al. 2011). There are several factors associated with the successful eradication of an invasive species (Myers et al. 2000, Simberloff 2009). These include an efficient and rapid response in order to tackle invasions when densities are still low and genetically constrained (Bax et al. 2002, Genovesi 2007, Mehta et al. 2007), control methods specific to the biology of the target species (Wotton et al. 2004), clear lines of authority to ensure accountability (Miller et al. 2004), and sufficient funding for such projects (Myers et al. 2000). Marine examples of each of these factors are discussed below.

Rapid response increases the likelihood of successful control of a species before extensive spread makes successful management unachievable (Bax et al. 2002, Wotton et al. 2004, Anderson 2005, Genovesi 2007), and this approach has been a vital component in successful management of marine incursions. For example, the invasive mussel *Mytilopsis* sp. that was discovered in an Australian yacht club, reached densities of 23,650 individuals per m^2 in under six months (Bax et al. 2002). The identification of this species spurred provincial and national government agencies into action immediately. The quick response by managers and the Australian government prompted the development of a national taskforce to identify potentially contaminated vessels that had since left the marina. Following a confirmed absence of 12 months, the *Mytilopsis* sp. eradication programme was declared a success. Similarly, upon the discovery of *Unidaria pinnatifida* on a sunken trawler near the Chatham Islands, an efficient rapid response by New Zealand authorities prevented the species from establishing (Wotton et al. 2004). The day after the species was discovered, it was assigned "Unwanted organism" status under the national Biosecurity Act of 1993 and thus bureaucratic delays did not hinder management action (Wotton et al. 2004). The algal species *Caulerpa taxifolia,* is well known for its spread throughout the Mediterranean Sea. However, as a result of its reputation in Europe, it had been placed on the US Federal Noxious Weed list (Anderson 2005). When the species was first detected in the United States in 2000, concerned stakeholders put pressure on the government to develop a containment and treatment plan within 17 days of discovery. As a result, the species was successfully eradicated from California over a period of three years (Genovesi 2007).

A second important component of successful eradication programmes is sound biological knowledge of the target species (Simberloff 2003, Wotton et al. 2004, Hopkins et al. 2011). The *U. pinnatifida* invasion discussed above was controlled with an integrated management plan designed to target the two life history stages of the species. Although sporophytes were visible to the naked eye, gametophytes were not and this necessitated a long monitoring period to ensure eradication. The species was declared eradicated three years after the treatment was initiated (Wotton et al. 2004). An alternative approach that also requires a clear understanding of the biology of the species concerned, involves the containment of a population below a critical density threshold. This tactic was used when a semi-submersible drilling rig in New Zealand was discovered heavily fouled with the brown mussel, *Perna perna*. Accordingly, an eradication programme was designed to reduce *P. perna* to a critical reproductive density threshold of one individual per 10 m^2 below which the population could not survive (Hopkins et al. 2011).

In many cases, an invasion can fall under the jurisdiction of different agencies or even countries and thus communication and cooperation between the various parties becomes critical. Following a large-scale intertidal survey in California, the Northern Atlantic fucoid, *Ascophyllum nodosum,* was detected (Miller et al. 2004). Several agencies co-operated in an eradication effort that involved the systematic manual removal of all plants and plant

fragments. The eradication was declared successful in 2004, following a year and a half of negative surveys (Miller et al. 2004).

Above all, eradication programmes require sufficient funding in order to be successful (Myers et al. 2000, Genovesi 2007). Usually government departments or conservation agencies cover the costs of management action, however, there are a few exceptions. When *U. pinnatifida* was introduced to New Zealand on a trawler, the vessels insurance company was held liable (Wotton et al. 2004). This is thought to be the first case worldwide of public liability insurance covering the management costs of an invasive species (Wotton et al. 2004).

South African marine invasions

Marine invasions in South Africa have followed the international trend with the number of recognised alien species increasing through time (Griffiths et al. 1992, Robinson et al. 2005) from the first discovery in 1846 (Mead et al. 2011a). The most recent review revealed the presence of 89 alien marine species in South Africa, of which 53 are invasive (Robinson et al. 2016). The relative proportions of the major groups include 28% Crustacea, 15% Cnidaria, 12% Mollusca, 10% Annelida and 10% Chordata. The invasive crustaceans include the global invader, *Carcinus maenas*, the European shore crab (Robinson et al. 2016). A spatial analysis of marine invasions in this region revealed that the majority have been noted on the temperate west coast of South Africa (Mead et al. 2011a). Most introductions are associated with harbours (Mead et al. 2011a), with only six invasive species widespread along the open coast (Robinson et al. 2016). The most important vectors for South African marine introductions are ballast water (38%) and ship fouling (48%) (Mead et al. 2011a). Of the South African ports, Table Bay harbour is recognised as the busiest, however traffic received here is considerably less than other international ports (Peters et al. 2017). For example, the annual traffic in the Cape Town port is approximately 33% compared to the most frequented port in the United States of America (Peters et al. 2017). In particular, harbours in area with low productivity and those associated with yachts are most likely to support higher numbers of alien species (Peters et al. 2017). Despite these studies acknowledging the scope of marine invasions, there has been no attempt to date in South Africa, or indeed the continent, to manage these species.

The ecology of *Carcinus*

The *Carcinus* genus belongs to the family Portunidae and is comprised of two species; the European shore crab, *C. maenas,* and the Mediterranean shore crab, *C. aestuarii.* The native range of *C. maenas* covers the eastern Atlantic Sea from Norway in the North to Mauritania

in the South (Carlton & Cohen 2003). *Carcinus aestuarii* is native to the Mediterranean Sea with the Strait of Gibraltar acting as a partial larval barrier for the species (Geller et al. 1997). It has been hypothesised that hybrids may exist in this zone (Geller et al. 1997, Clark et al. 2001), however no evidence of this has been detected to date. *Carcinus aestuarii* is not as widespread as its congener and thus the majority of published literature focuses on *C. maenas*.

In its native range, *C. maenas* inhabits sheltered habitats. Megalopae and juveniles are often found in algal or mussel beds, in sediment, under rocks or crushed shell deposits in the intertidal zone (Behrens Yamada 2001, Jensen et al. 2002). This crab is most often found from the high tide mark to a depth of 6 m, but has been recorded at depths of 60 m (Grosholz & Ruiz 1996). Within its native range, three size classes of *C. maenas* have been identified with distinct habitat preferences. Crabs that are < 34 mm in carapace width tend to occupy the intertidal zone, while crabs which have a carapace width > 40 mm predominantly live subtidally with some foraging in the intertidal zone. The third group of crabs, comprised of the oldest individuals are exclusively subtidal (Crothers 1968). *Carcinus maenas* is known to migrate up and down the shore with the changes of the tide and larger specimens cover a greater range (Naylor 1962).

Adult *C. maenas* can survive at temperatures between 0 and 35.8°C (Hidalgo et al. 2005) but the optimal range for reproduction is between 3 - 26°C (Grosholz & Ruiz 2002). Larval development occurs between 9 - 22.5°C (Dawirs et al. 1986, de Rivera et al. 2007). The optimal salinity for adult crabs is between 10 and 31‰ (Broekhuysen 1936) while minimum salinities of 20‰ are essential for larval development (Anger et al. 1998). The European shore crab is able to tolerate low (up to 3 kPa) partial pressure of oxygen depending on the salinity (Legeay & Massabuau 2000) and can survive up to seven days out of water (C. Mabin pers. obs.).

The maximum size of an adult *C. maenas* carapace recorded to date is 100 mm for males and 79 mm for females (Behrens Yamada 2001). However, there is some global variability in size and growth rates (see Behrens Yamada 2001). The lifespan of *C. maenas* is 3-6 years (Berrill 1982, Grosholz & Ruiz 1996, Behrens Yamada 2001). Females produce one or two clutches of eggs a year, with a maximum of 185,000 eggs at a time (Broekhuysen 1936). Laboratory experiments have demonstrated that at 15°C, female *C. maenas* reach puberty moult at a mean carapace width of 46 mm (range 34-55 mm) (Mohamedeen & Hartnoll 1989). However, at warmer temperatures, the crabs are able to reproduce at a smaller size, i.e. mean carapace width of 42 mm (range 27-49 mm) (Mohamedeen & Hartnoll 1989).

Carcinus maenas have six larval stages that comprise one protozoea stage, four pelagic zoea stages and one postlarval (megalopae) stage (Behrens Yamada 2001). Larval development lasts 40 days under laboratory conditions (Williams 1967). Generally, larvae are found to a depth of 60 m in coastal waters, at an average depth of 20-25 m during the day and 30-45 m

at twilight (Queiroga 1996). Off the coast of Portugal, *C. maenas* larvae were found up to 45 km offshore, with the majority between 15 and 20 km from the shore (Queiroga 1996). Megalopae utilize selective tidal stream transport to return inshore to settle and metamorphose into juvenile crabs (Queiroga 1998). Megalopae appear to select complex habitats for settlement, preferentially occupying algae, eelgrass, or mussel beds close to bare sand (Moksnes 2002). In its native range, in Sweden, settlement peaks have been recorded for *C. maenas* with a total recruitment of 3.7 million megalopae over a seven-day period (Moksnes 2002).

Carcinus maenas is omnivorous and preys on organisms from at least 158 genera, from five plant and protist phyla and 14 animal phyla (Cohen et al. 1995). Notably, prey includes bivalves, gastropods, crustaceans and fishes, some of which are commercially important. Planktonic larvae consume phytoplankton primarily (Harms et al. 1994), with early stage juveniles feeding on detritus and infauna while adults are omnivorous (Behrens Yamada 2001, Johnston & Freeman 2005). This broad diet may well be key to the crab's global success. *Carcinus maenas* have been reported digging for prey in sediment, to a maximum depth of 15 cm, which may have consequences for benthic infaunal communities (Cohen et al. 1995).

***Carcinus* invasions**

Carcinus maenas is a successful global invader with established populations in Argentina (Hildago et al. 2005), Australia (Fulton & Grant 1900), Canada (Paille et al. 2006), South Africa (Joska & Branch 1986) and in the United States of America (Cohen et al. 1995). In addition, single specimens have been reported from the Red Sea (prior to 1817), Brazil (1857), Panama (1866), Sri Lanka (1866-67), Hawaii (1873), Madagascar (1922), Myanmar (1933) and Pakistan (1971) (Carlton & Cohen 2003). *Carcinus aestuarii* has established in Japan (Sakai 1986). In its non-native range, *C. maenas* has been found in sheltered coastal and estuarine habitats as well as semi-exposed rocky coasts (Behrens Yamada 2001).

In Australia and South Africa range expansion by *C. maenas* has been surprisingly slow (Thresher et al. 2003). This could be attributed to localised recruitment, especially within bays (Byers & Pringle 2006). It should be noted that on the east coast of North America, where the population appeared to have reached its maximum extent, a new genetic strain of *C. maenas* led to considerable range expansion (Blakeslee et al. 2010). *Carcinus maenas* has limited ability to resist hydrodynamic forces compared to native crab species in South Africa and therefore cannot survive in intertidal areas with high levels of water movement (Hampton & Griffiths 2007). The South African coastline has high levels of wave exposure (Sink et al. 2012) relative to many other regions of the world and this might explain the

apparently restricted spread of *Carcinus* along the open coast compared to its other invasive ranges.

The realised impacts of *C. maenas* are challenging to determine as there is very little information available regarding the pre-introduction state of currently invaded regions. The crab is predicted to have ecological impacts across much of its invasive range, with some commercial species also affected (Welch 1968, Behrens Yamada 2001, Grosholz & Ruiz 2002, Walton et al. 2002). In some cases, *C. maenas* predation appears to have resulted in evolutionary change with thickening of shells of several snail species (Freeman et al. 2013), as well as the adoption of avoidance strategies in clams such as greater burrowing depth and longer siphons (Whitlow et al. 2003, Whitlow 2010).

***Carcinus* in South Africa**

The introduction of *C. maenas* to South Africa most likely occurred through the fouling of oilrigs, ship hulls or ballast water discharge (Mead et al. 2011b). *Carcinus maenas* was first detected in Table Bay in 1983 (Joska & Branch 1986). However, subsequent genetic analyses revealed haplotypes of both *C. maenas* and *C. aestuarii* to be present in the country (Geller et al. 1997). A later study using morphometric characteristics found only *C. maenas* present in the Table Bay and Hout Bay populations (Robinson et al. 2005). This was also the first description of the Hout Bay population. In a later genetic study using the same samples as collected by Geller et al. (1997), evidence of hybridisation and mitochondrial introgression between the two species were found (Darling et al. 2008). This rare phenomenon was confirmed in the Japanese *C. aestuarii* populations following further sampling (Darling 2011).

There are several factors associated with *C. maenas* that could potentially threaten South African marine biodiversity. There appear to be no native comparative species (Le Roux et al. 1990) that might offer competitive resistance and thus control the spread of this crab. There thus appears to be an open niche with potentially limited biotic resistance. In addition, unlike its native range, *C. maenas* in South Africa appear to be completely parasite free (Zetlmeisl et al. 2011). As an aggressive predator, with a broad diet, there are considerable ecological impacts suggested for this species. A study was undertaken early in the invasion to predict potential impacts but this was limited to impacts within the intertidal environment (Le Roux et al. 1990). However, it is unclear what impacts the crab currently has within South Africa. Considering that *Carcinus maenas* has a pelagic larval life history stage that can result in the transport of larvae up to 45 km offshore (Queiroga 1996), notable potential exists for current-induced spread. In addition, the extensive global distribution of this species combined with international and domestic vectors (with limited preventative measures in place) increases the probability of further spread.

Rationale for this study

Carcinus maenas is listed as an invasive species requiring control under the National Environmental Management: Biodiversity Act 2004. However, there are many invasive species that are recognised as management targets in South Africa and therefore thorough assessments of the threat and the feasibility of management are needed to prioritise limited resources. Triage is a well-recognised technique used to assist with decision making and prioritising medical emergencies (Kennedy et al. 1996). This concept has also been adopted in environmental management when dealing with natural disasters (Bottrill et al. 2008) and has more recently been applied to invasive species management (Downey et al. 2010, Murphy et al. 2014). By prioritising the most severe cases where management interventions are most likely to succeed, resources can be optimally utilised.

In order to fulfil the legal mandate and assess the appropriate priority level for *Carcinus*, a comprehensive analysis focusing on several aspects of the *Carcinus* invasion in South Africa was necessary. Although there have been a few studies conducted to date on *Carcinus* in South Africa (Le Roux et al. 1990, Geller et al. 1997, Robinson et al. 2005, Darling et al. 2008), these are now outdated offeringinsufficient to support management decisions. The first distribution survey and the only impact assessment were undertaken in 1984, a year after the crab was first detected (Le Roux et al. 1990). In 1996, genetic samples were collected for global analyses of the *Carcinus* genus (Geller et al. 1997, Darling et al. 2008).

This thesis aimed to review the current distribution and status of *Carcinus* in South Africa, and investigate options for management in this region. The impacts of this invader at multiple trophic levels and the genetic patterns underlying the invasion history were assessed to determine the severity of impacts and the potential for re-introduction. The specific aims addressed were as follows:

1) To determine the current distribution and population status of *Carcinus* in South Africa;
2) To undertake the first management programme targeting a marine invasive species in Africa in order to assess the feasibility of eradicating the species from South Africa;
3) To determine the ecological impacts of *Carcinus* in South Africa;
4) To estimate the most likely invasion scenarios for *Carcinus* and determine whether the two South African populations represent independent introductions.

CHAPTER 2. THE CURRENT DISTRIBUTION AND POPULATION STATUS OF *CARCINUS* IN SOUTH AFRICA

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Abstract

With the continued spread of invasive species globally, it is essential that the population status and distribution of such species be updated regularly to allow for effective management. The crab genus, *Carcinus*, has been present in South Africa since 1983, when the first individuals were detected in Table Bay harbour. Over the last thirty years, three snapshot surveys (in 1990, 2002 and 2013) have been conducted to assess the distribution of the crab in the Western Cape, with recent port surveys unable to detect *Carcinus* outside of this region. The present study is the most comprehensive to date, providing an update on the range, abundance and demographics of the species. An intensive survey of all known populations (in intertidal and subtidal habitats) was conducted from 2014 to 2015. Intertidal surveys involved thirty minutes of searching by two researchers at low tide while the subtidal environments along the coast were surveyed by divers. *Carcinus* was not detected at the intertidal sites, except for Sea Point and no crabs were found during subtidal surveys along the open coast. Subtidal harbour populations were recorded in the Cape Town harbours of Table Bay and Hout Bay. Table Bay was surveyed monthly for one year using baited traps, crab condos and postlarvae settlement collectors in order to assess size distributions and reproductive seasonality of the crab. Reproductive females were found throughout most of the year. These results indicate that the harbour populations could be targeted by control programmes if required, with no strong evidence to support the initiation of management action during a particular season. Regular monitoring is recommended at sensitive sites which represent suitable habitat for *Carcinus* as early detection at these locations could support management of these incursions.

Introduction

The global spread of marine invasive species is predicted to continue increasing with time (Cohen & Carlton 1998, Bax et al. 2003, Clarke Murray et al. 2014). Such species can be associated with ecological and socio-economic impacts in the invaded areas (Gurevitch & Padilla 2004, Lovell et al. 2007, Molnar et al. 2008, Nentwig et al. 2016). In order to reduce

these impacts but with limited resources available, species of concern need to be identified and prioritised (Downey et al. 2010, Faulkner et al. 2014). Several countries have developed legislation listing invasive species that are currently present as well as those prohibited from import (Simberloff et al. 2005, Randall et al. 2008, McGeoch et al. 2010, McGeoch et al. 2012, Faulkner et al. 2014). The current distribution and population status of species must be regularly assessed in order to implement effective management and monitoring plans that then inform legislation (McGeoch et al. 2012). For example, the revision of species lists may be required if a listed species is no longer present (Mabin et al. 2015), or if the ranges of known alien species change (Robinson et al. 2005).

The European shore crab, *Carcinus maenas*, features on the International Union for the Conservation of Nature Global Invasive Species Database list of '100 of the World's Worst Invaders' (GISD 2017). This marine crab, belonging to the Family Portunidae, has a widespread global non-native distribution, having been recorded in Australia (Fulton & Grant 1900), South Africa (Joska & Branch 1986), the USA (Cohen et al. 1995), Argentina (Hildago et al. 2005) and Canada (Paille et al. 2006). Many of these countries have recorded established populations (Fulton & Grant 1900, Cohen et al. 1995, Hildago et al. 2005, Robinson et al. 2005, Paille et al. 2006) and several of these have reported ecological and economic impacts (Ropes 1968, Davis et al. 1998, Grosholz et al. 2000, Lovell et al. 2007, de Riviera et al. 2011, Grosholz et al. 2011, Malyshev & Quijon 2011, Freeman et al. 2013, Mach & Chan 2013, Whitlow et al. 2010). Invasive populations of *C. maenas* have been extensively studied and found to have caused declines in abundance of the native clams, *Nutricola tantilla* and *N. confusa*, as well as the shore crab, *Hemigrapsus oregonensis* in California (Grosholz et al. 2000), while in Nova Scotia, a significant reduction in biomass of eelgrass (*Zostera marina*) was attributed to the presence of *C. maenas* (Malyshev and Quijon 2011). In addition, the crab has caused morphological and behavioural changes in the native clam, *Mya arenaria* (Whitlow 2010) and whelk, *Haustrum vinosum* (Freeman et al. 2013). The economic impacts of this species are felt primarily by mariculture operations, especially those growing bivalves in the benthic environment. These cultured species are particularly at risk from *C. maenas* predation (Ropes 1968, Lovell et al. 2007, Grosholz et al. 2011, Mach & Chan 2013). Based on these impacts of *C. maenas* in other invaded regions, the species has been prioritised as a target for control under South Africa's National Environmental Management: Biodiversity Act of 2004 (NEM:BA) (i.e. listed as a category 1b species in the Draft Amendments to the Alien and Invasive Species lists, 2015).

Carcinus maenas was first recorded in South Africa in 1983 (Joska & Branch 1986). Since the initial discovery, several surveys have been conducted to assess the species distribution (Le Roux 1990, Robinson et al. 2005, Jooste 2013) (Figure 2.1). *Carcinus* has been found both intertidally and subtidally, however, it thrives in sheltered bays and harbour environments (Hampton & Griffiths 2007). As a portunid crab (i.e. a swimming crab), *C. maenas* has a light carapace which in combination with the reduced length and strength of its rear dactyls

(Hampton & Griffiths 2007), contributes to its inability to secure a grip on rocks under high wave action.

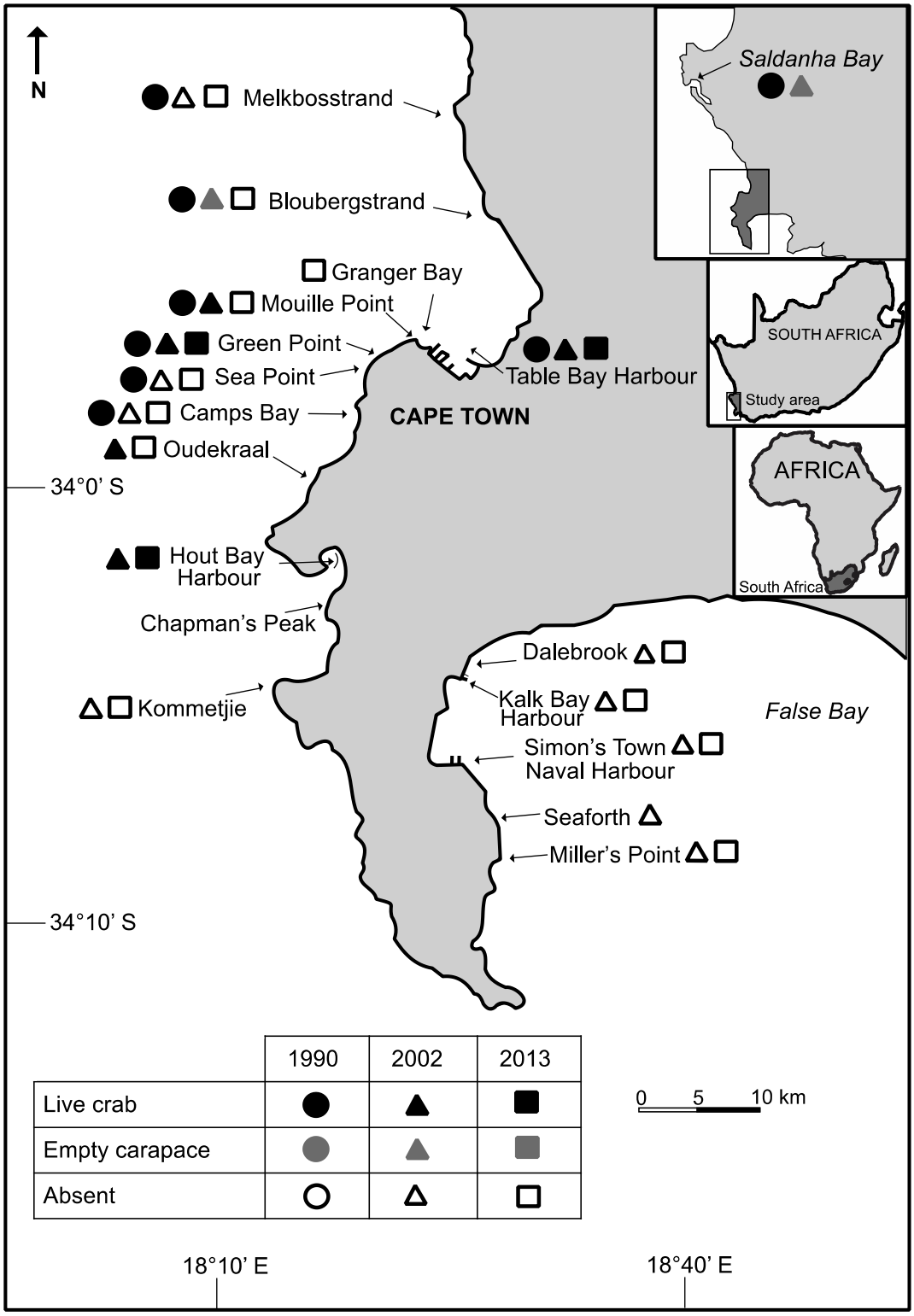

Figure 2.1. Map of the greater Cape Town area and Cape peninsula, showing intertidal and subtidal sites surveyed by Le Roux et al. (1990) in 1990, Robinson et al. (2005) in 2002 and Jooste (2013) in 2013 and the present study.

To date, the only populations recorded in South Africa are in Table Bay and Hout Bay (Le Roux et al. 1990, Robinson et al. 2005), with a few crabs detected at other sites close to Cape Town (Robinson et al. 2005). Surveys of ports and small harbours across South Africa have failed to detect *Carcinus* anywhere else in the country (Awad et al. 2004, Hutchings et al. 2006, Laird et al. 2013, Peters et al. 2014, Peters et al. 2017). A mating pair was discovered in 1990 in Saldanha Bay (Le Roux et al. 1990), however, since then only one empty carapace and no live animals have been discovered (Robinson et al. 2004), despite annual monitoring conducted on the subtidal and intertidal communities in the bay (Clark et al. 2015). Langebaan Lagoon, situated within the Saldanha Bay system on the west coast, is considered vulnerable to *Carcinus* invasion due to its sheltered nature and its proximity to a commercial port (Robinson et al. 2004). The Lagoon lies within the West Coast National Park, which also contains a wetland of international importance as recognised by the Ramsar Convention. This high marine diversity could be impacted if the crab were to invade (Robinson et al. 2004).

The first estimates of population size of this species were conducted in 2002 by Robinson et al. (2005). The Table Bay population was estimated to comprise 133,500 individuals (95% CI: 111,000 - 156,100) and Hout Bay, 9,200 individuals (95% CI: 4,900 – 13,500) (Robinson et al. 2005). Follow-up surveys in 2013 recorded populations of 164,200 (95% CI: 153,900 - 174,500) in Table Bay and 6,500 (95% CI: 700 - 12,600) in Hout Bay, suggesting that these populations had not changed significantly in size or density and appeared to be stable (Jooste 2013). At the time, intertidal populations appeared to be decreasing, with *C. maenas* only being recorded at a single site (Green Point) (Jooste 2013). It is notable that these previous population studies did not consider seasonal patterns of abundance and distribution, only providing a snapshot of the status of this invasive crab. However, in its native range, the abundance and distribution of *C. maenas* varies with season (Naylor 1962, Crothers 1968), and this pattern may also occur in South Africa.

This chapter aims to update our knowledge of the distribution, population status and demographics of this invasive crab in South Africa and offer insight into the spatial and temporal variability of these population parameters. In particular, the following hypotheses were tested: (1) *Carcinus* would support intertidal and subtidal populations at sites close to Table Bay and Hout Bay harbours despite declining at more distant sites over the last decade; (2) breeding within this species would be restricted during summer when intense upwelling reduces water temperatures below the critical limit for moulting and reproduction. By addressing these aims this chapter will provide the necessary biological information required to support management decisions regarding *Carcinus* in this region.

Methods

This study involved both regular intertidal and single subtidal surveys along the coast, as well as an intensive survey of the Table Bay population using postlarvae settlement collectors, crab condos and baited traps. Intertidal and subtidal surveys followed the same methods as previous studies (Robinson et al. 2005, Jooste 2013).

Intertidal surveys

Six sites at which *Carcinus* was previously recorded (Figure 2.1) were monitored for one year. Surveys were conducted on a monthly basis between March 2014 and February 2015 (Table 2.1). An additional four sites where *Carcinus* had not been reported before, but which appeared to support suitable habitat, were surveyed quarterly. When *Carcinus* individuals were recorded at Sea Point, monitoring was extended at this site, although surveys took place quarterly from March 2015 to February 2016. During surveys, two researchers searched each site for 30 minutes at spring low tides. Suitable crab habitat was searched with particular attention paid to areas under algal fronds, boulders and in rock crevices. The number of *Carcinus* observed was recorded and a note made of any gravid (i.e. egg-carrying) females.

Table 2.1. Coastal intertidal and subtidal site details for distribution surveys. Sites previously known to support *Carcinus* are indicated with a [#].

Site	Description	Frequency	GPS Co-ordinates
Melkbosstrand [#]	Intertidal	Monthly	33.7646 18.4347
Blouberg [#]	Intertidal	Monthly	33.8034 18.4608
Mouille Point [#]	Intertidal	Monthly	33.8990 18.4085
Green Point [#]	Subtidal	Single	33.9060 18.3973
Sea Point [#]	Intertidal	Monthly→Quarterly	33.9124 18.3871
Camps Bay [#]	Intertidal	Monthly	33.9529 18.3740
Oudekraal North [#]	Intertidal/Subtidal	Quarterly/Single	33.9814 18.3597
Hout Bay [#]	Subtidal	Single	34.0523 18.3492
Chapmans Peak	Intertidal	Monthly	34.0646 18.3681
Kommetjie	Intertidal	Quarterly	34.1418 18.3217
Seaforth	Intertidal	Quarterly	34.1934 18.4467
Dalebrook	Intertidal	Quarterly	34.1243 18.4527

Subtidal surveys of natural habitat

Single subtidal surveys were conducted at three sites along the coast between April and June 2014 (Table 2.1). The Hout Bay survey was conducted in the Bay itself, outside of the harbour environment, while the Oudekraal and Green Point surveys took place along the open coast. Three replicate transects at average depths of 5, 10 and 15 m were surveyed by

scientific SCUBA divers for a length of 50 m parallel to the shore. The number of *Carcinus* detected within 1 m either side of each transect line was recorded.

Harbour surveys: population structure and reproductive cycle

Baited traps were used to detect *Carcinus* in Hout Bay and Table Bay harbours in April 2014. The population structure was assessed on a monthly basis from April 2014 to March 2015 in the Royal Cape Yacht Club in Table Bay harbour. In order to assess all life stages of *Carcinus*, three trapping techniques were adopted: 1) artificial settlement collectors for postlarvae; 2) unbaited crab condos for smaller individuals < 50 mm; and 3) baited traps for crabs of all size classes.

1) Postlarvae settlement collectors

Postlarval abundance was estimated using artificial settlement collectors (Figure 2.2). Collectors were constructed using a PVC pipe (11 cm diameter by 30 cm length) wrapped in a quilt batting sleeve (a surface area of 0.10 m^2). This coarse material had a complex surface structure which results in the passive settlement of postlarvae, with abundance correlated with those in the water column (Moksnes & Wennhage 2001). A float was attached to the top and a weight at the bottom to suspend the structure vertically just below the water surface (van Montfrans et al. 1995). Four replicate collectors were soaked during the day for 6 hours and retrieved at high tide, as settlement densities are highest during the daytime flood (Moksnes et al. 2003). Following retrieval, sleeves were soaked in a 20 l bucket of fresh water for a minimum of 30 minutes to encourage the detachment of postlarvae (Moksnes et al. 2003). The water was then filtered through a 750 µm sieve and viable postlarvae enumerated. Sleeves were rinsed and dried in the sun prior to re-deployment.

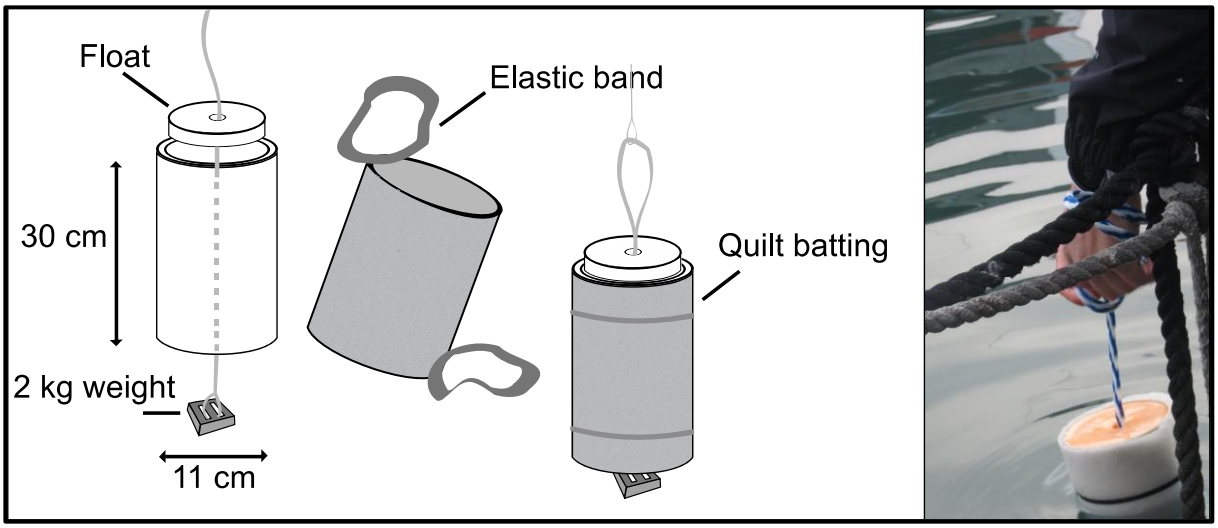

Figure 2.2. Artificial settlement collectors used to collect *Carcinus* postlarvae.

2) Unbaited crab condos

The densities of small (< 50 mm carapace width) crabs were estimated using crab 'condos' (Hewitt & McDonald 2013) (Figure 2.3). These unbaited traps were constructed of nine PVC pipes with an internal diameter of 45 mm and a length of 250 mm (with a total volume of 3.6 l per condo). Four replicates were deployed for a period of 24 hours approximately 40 m apart on a soft-sediment benthic surface, with an average depth of 3 m. Upon retrieval, the carapace width of each crab was measured using vernier callipers, crabs were sexed and female gravid status noted. All crabs were returned to the water following examination.

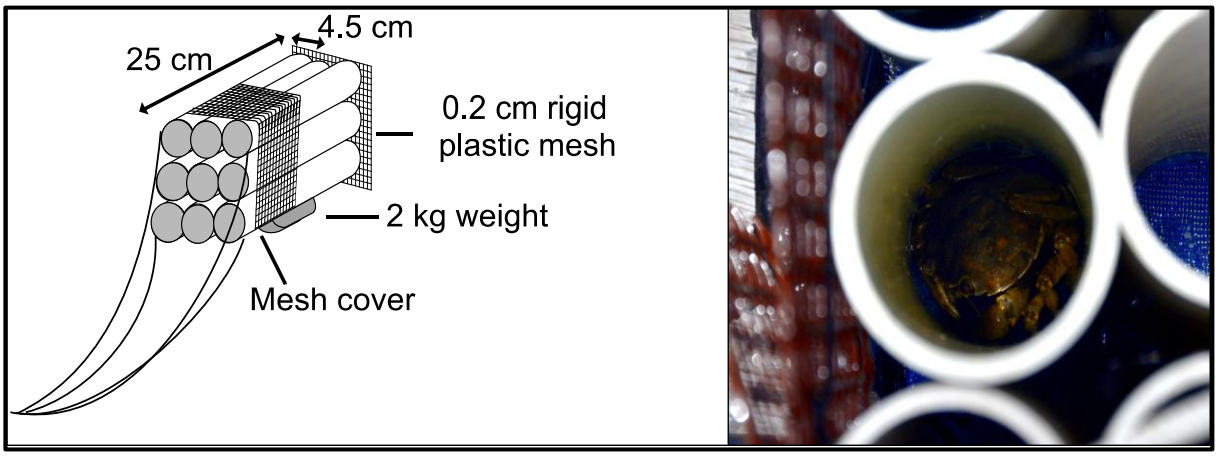

Figure 2.3. Crab condo used to collect *Carcinus* crabs < 50 mm (adapted from Hewitt and McDonald 2013).

3) Baited traps

Crabs were collected in four replicate two hour soaks using crab traps (750 x 250 x 250 mm; total volume of 47 l, with a flexible entrance of 100 mm diameter) and baited with 200 g of crushed sardines (Figure 2.4). See Chapter 3 for details regarding soak time. Traps were deployed on a soft-sediment benthic surface approximately 40 m apart at an average depth of 3 m. Upon retrieval, the carapace width of each crab was measured using vernier callipers, and then sorted according to the following size classes: Small (<41 mm); Medium (41-60 mm), Large (61-80 mm) and Extra-large (>80 mm). Individuals were sexed and female gravid status recorded. All crabs were returned to the water following data collection so that future monitoring was not affected.

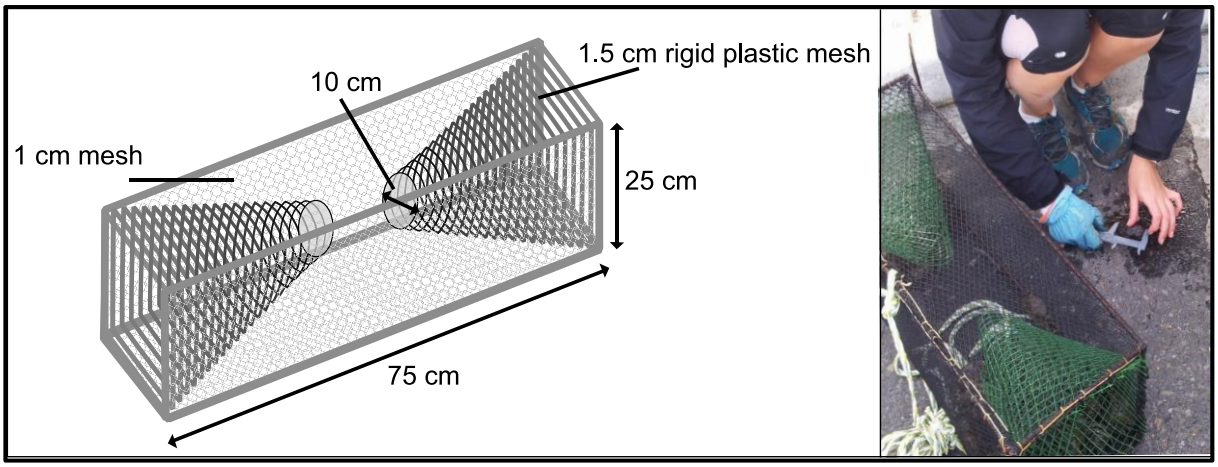

Figure 2.4. Baited trap used to collect individuals of all size classes.

Data analysis

All statistical analyses were conducted in R (version 3.2.3, R core team 2016).

Postlarvae counts were analysed with respect to month to determine whether there was a seasonal peak in postlarval settlement using a Pearson's Chi Squared test. Trigonometric curves were fitted to the crab condo data to account for the recurrent pattern associated with seasonal variation using the following linear model:

$$\gamma = \text{count} \sim \sin(2 \times \pi \times (\frac{month}{12})) + \cos(2 \times \pi \times (\frac{month}{12}))$$

whereby each month between April 2014 and March 2015 was numbered in chronological order. Crab condo catches in Table Bay harbour were then examined with respect to month (12 levels: April 2014 to March 2015) and sex (two levels: male and female) in a two-factor generalised linear model (GLM) with a Poisson error distribution.

Trigonometric curves were fitted to the counts of *Carcinus* in baited traps (using the model described above) and then compared among months (12 levels: April to March) and sexes (two levels: male and female) in a two-factor GLM, using a Poisson error distribution. Similarly, the number of crabs in the various sizes class (four levels: ≤ 40, 41-60, 61-80 and ≥ 81 mm carapace width) were compared among months (12 levels: each month from April to March) in a two-factor GLM, again with a Poisson error distribution.

Finally, the number of gravid and non-gravid females were compared across months (12 levels: from April to March) using a one-factor GLM with a binomial distribution.

Results

Intertidal surveys

Carcinus were not found during any of the intertidal surveys, with the exception of Sea Point. At this site, in March 2014, two males and one female crab were found and in February 2015, one male was found. As a result, this population was surveyed quarterly for another year, however, no further individuals were found.

Subtidal surveys of natural habitat

The subtidal surveys in Green Point, Oudekraal and outside Hout Bay harbour detected no *Carcinus* individuals. However, two native crab species, the Cape rock crab, *Plagusia chabrus,* and the Shore crab, *Cyclograpsus punctatus,* were found at these sites.

Harbour surveys: population structure and reproductive cycle

1) Postlarvae settlement

Carcinus postlarvae were not detected in Table Bay harbour over the sampling period.

2) Crab condos

In Table Bay, the mean number of male and female crabs caught varied with month with no crabs caught in April, October, February and March (Figure 2.5). Neither of the trigonometric curves were a good fit for male crabs (df = 1; sin: z = 1.383, p = 0.167; cos: z = 0.200, p = 0.841) and only the sine curve was significant for females (df = 1; sin: z = 2.265, p

=0.024; cos: z = –1.271, p = 0.204). The absence of crabs in late summer (February to March) and in October was notable.

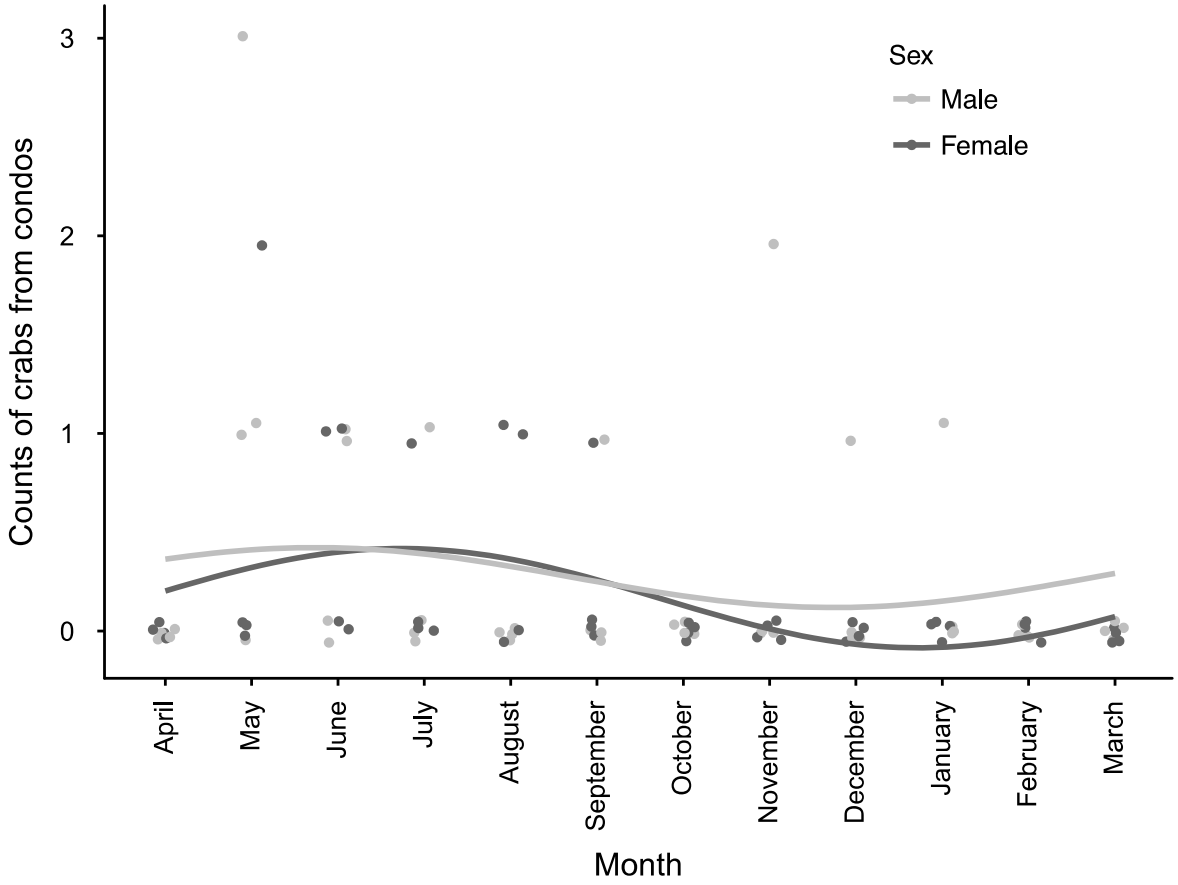

Figure 2.5. Jitterplot showing seasonal patterns in the abundance of male and female crabs (< 50 mm) in unbaited crab condos deployed in Table Bay harbour. Circles represent the number of crabs caught per sex per trap while lines represent trigonometric model predictions. The sine and cosine terms were not significant for the male models (p > 0.05). The cosine term was not significant for females (p > 0.05), however the sine term was significant (p = 0.024).

3) Baited traps

Trapping confirmed the presence of crabs in both harbours. In Table Bay, the highest total catch in one month was recorded during June, with 286 individuals captured. The number of each sex per month was well represented by trigonometric curves with both sine and cosine terms significant for males (df = 1; sin: z = 9.97, p <2 x 10^{-16}; cos: z = -5.40, p = 6.77 x 10^{-8}) and females (df = 1; sin: z = 6.99, p = 2.6 x 10^{-12}; cos: z = -4.55, p = 5.3 x 10^{-6}) (Figure 2.6).

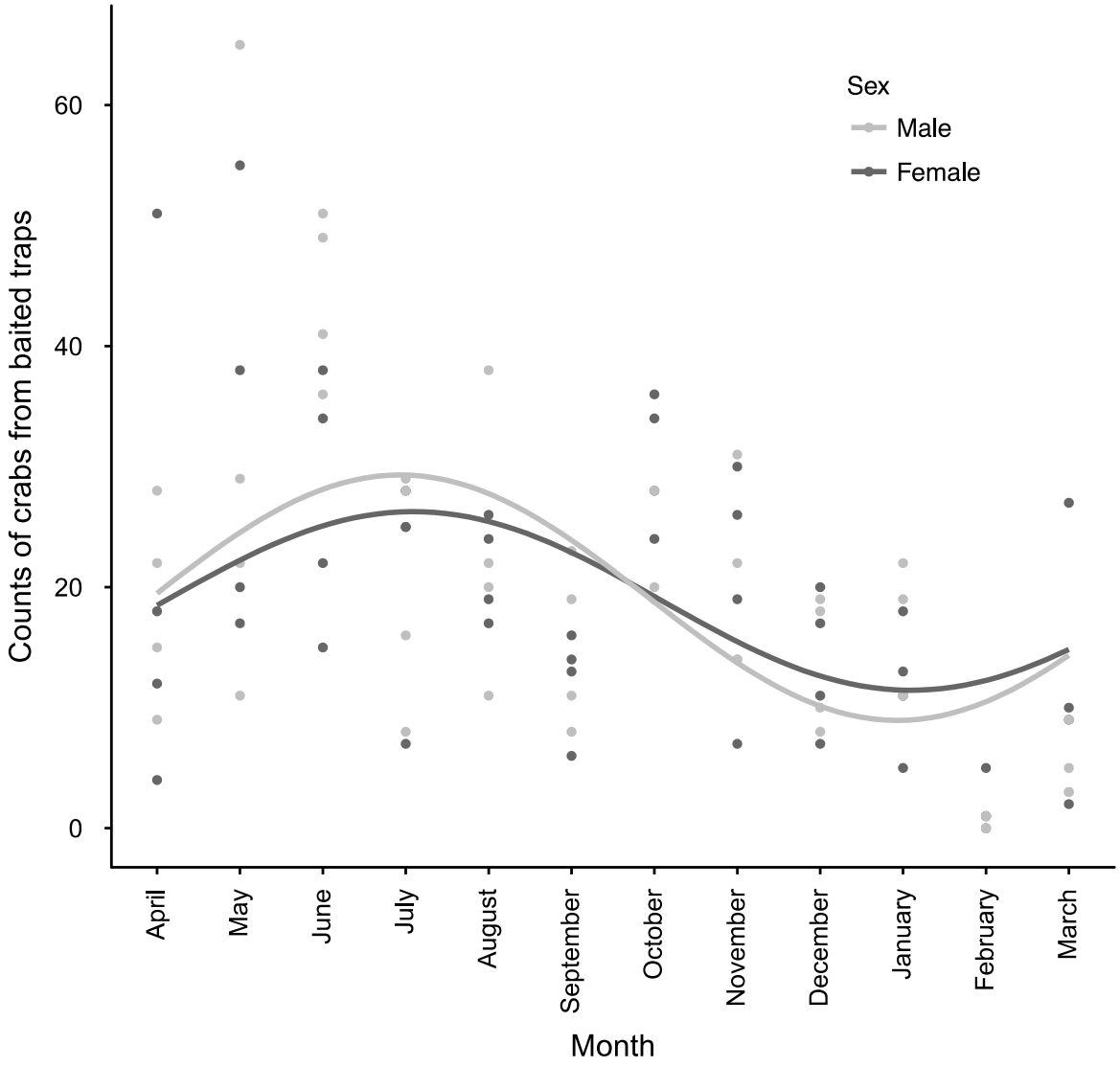

Figure 2.6. Seasonal patterns in the abundance of male and female crabs (all size classes) in baited traps deployed in Table Bay harbour. Circles represent the number of crabs caught per sex per trap while lines represent trigonometric model predictions. The sine and cosine terms were significant for both the male and female models ($p < 0.001$), with no effect of sex ($p = 0.76$).

When considering catches by size class, the trigonometric curve fitted the data well for medium crabs which was the dominant size (Figure 2.7), with both the sine and cosine terms being significant (df = 1; sin: z = 9.91, $p < 2.0 \times 10^{-16}$; cos: z = -5.19, $p = 2.1 \times 10^{-7}$). The second best fitting model was that for large crabs with both sine and cosine terms significant (df = 1; sin: z = 4.83, $p = 1.4 \times 10^{-6}$; cos: z = -3.522, $p = 4.3 \times 10^{-4}$). Both the sine and cosine terms for small crabs were significant (df =1; sin: z = 5.20, $p = 2.0 \times 10^{-7}$; cos: z = -2.38, $p = 0.017$). It was notable that a very low number of small crabs were recorded between December 2014 and March 2015. Extra-large crabs were rarely caught in baited traps with only the cosine term being significant ((df = 1; sin: z = 1.32, $p = 0.19$; cos: z = -

2.22, p = 0.027) which may explain the poor-fitting model (Figure 2.7).

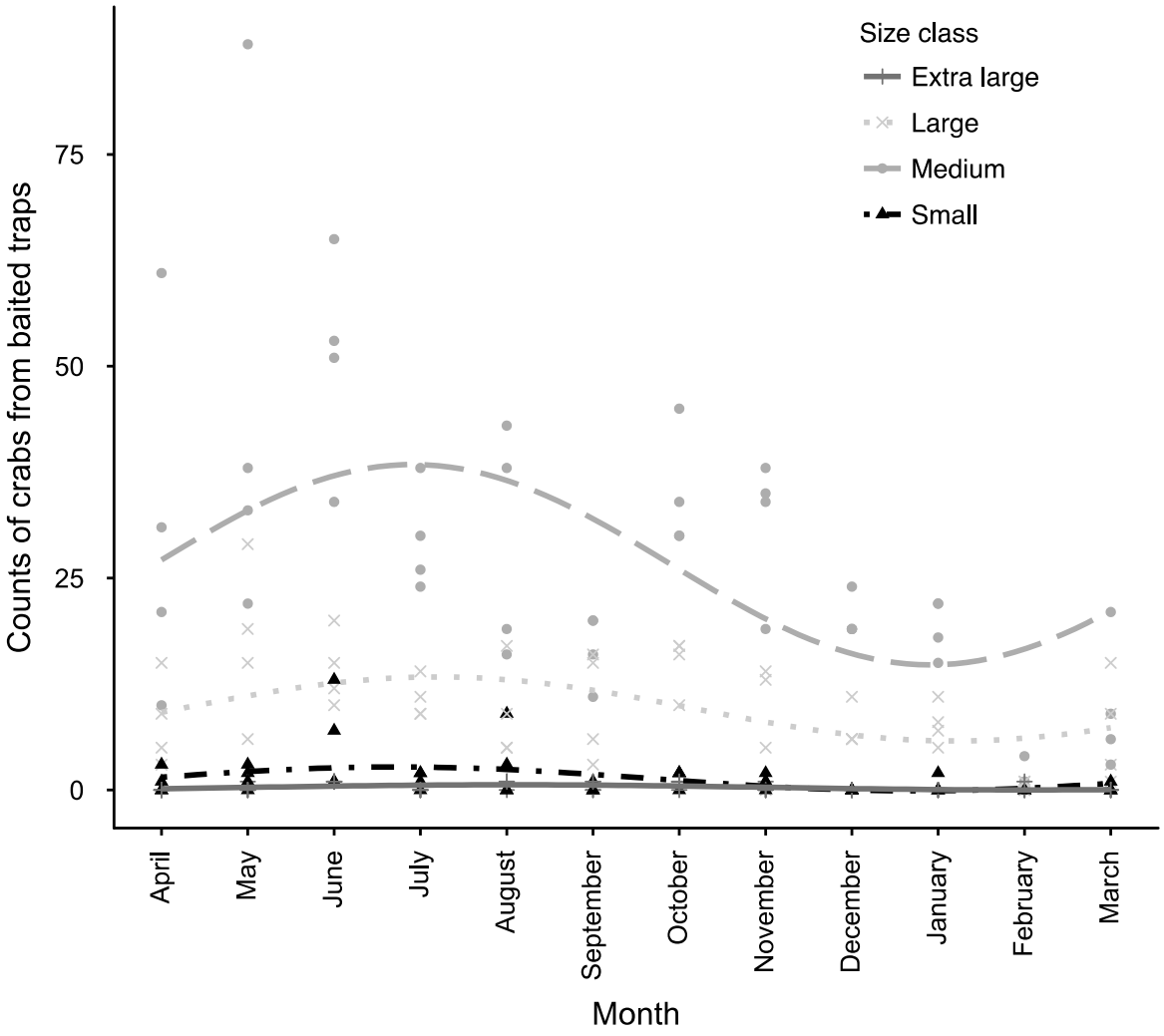

Figure 2.7. Seasonal patterns in the abundance of *Carcinus* in different size categories captured using baited traps in Table Bay, South Africa. The symbols represent the counts for each size class per trap while lines denote trigonometric model predictions. Size classes correspond to the following carapace widths: Small (< 41 mm); Medium (41-60 mm), Large (61-80 mm) and Extra-large (> 80 mm). Significant differences were detected among all size classes (p < 0.001) and the sine and cosine terms were significant for all models (p < 0.05), except for the sine term for the extra-large size class (p = 0.19).

While the proportion of gravid females captured in the baited traps peaked in October, no statistical effect of month was detected (X^2= 96.0, df = 88, p = 0.26) (Figure 2.8).

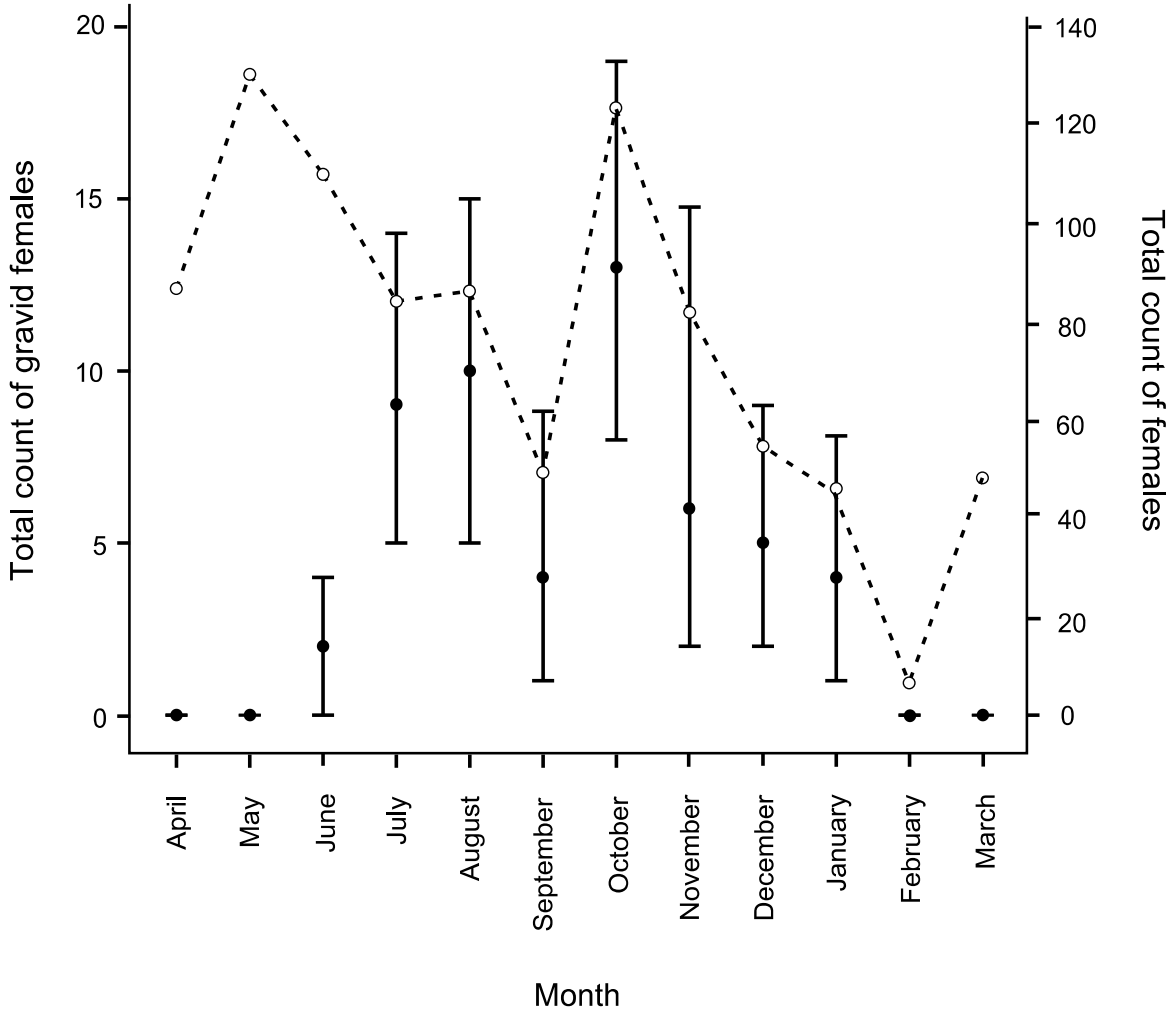

Figure 2.8. Seasonal patterns in the abundance of gravid female and total female *Carcinus* captured using baited traps in Table Bay, South Africa. The counts of gravid females (closed circles) per month are summed over the four replicates and displayed with 95% confidence intervals. These intervals are calculated using a binomial distribution of gravid females relative to non-gravid females. The abundances of total females per month are shown as open circles connected with a dashed line for clarity.

Discussion

In order to develop management plans for *Carcinus* (see Chapter 3 for further details), it was essential to establish the current distribution and population status, using the same methods as previous studies (Robinson et al. 2005, Jooste 2013). Following the trend documented by others over the last decade (Robinson et al. 2005, Jooste 2013), this study found that the species has declined in abundance intertidally and subtidally in undisturbed habitats along the west coast of South Africa. Sea Point was the only intertidal site which was found to support European shore crabs, albeit in very low numbers. In 2002, subtidal

populations were detected in Green Point, Oudekraal and outside Hout Bay harbour during single surveys (Robinson et al. 2005). However, this study has demonstrated that these populations might no longer exist and that the intertidal range of species appears to be retracting. This invasive crab is currently confined to two large breeding populations in Table Bay and Hout Bay harbours, with no evidence of range expansion since the surveys conducted in 2002. These findings were partly unexpected as it had been hypothesised that *Carcinus* would support intertidal and subtidal populations at sites close to Table Bay and Hout Bay harbours. It should be noted, however, that this the declining abundance might reflect temporal variation in abundance or recruitment rather than a directional trend, as considerable variation has been observed temporally in other *Carcinus* populations previously (Behrens Yamada et al. 2015).

Over the last two decades, the large commercial ports in South Africa have been surveyed for marine invasive species including Saldanha Bay (Awad et al. 2004), Port Elizabeth (Hutchings et al. 2006) and Richards Bay (Laird et al. 2013). More recently, eight harbours in the Western Cape were surveyed (Peters et al. 2014) and this was expanded to give a comprehensive update of all South African harbours (including international ports, small fishing harbours and regional marinas) in 2015 (Peters et al. 2017). None of these surveys (excluding those conducted in Table Bay and Hout Bay) detected *Carcinus*.

The inability of *Carcinus* to withstand the high wave exposures experienced on the shoreline of the west coast has led to the prediction that invasions of this species would be limited to protected harbours and bays in South Africa (Hampton & Griffiths 2007). This could at least partially explain the failure of the crab to maintain intertidal and open coast shallow subtidal populations, while large harbour populations persist. Given the predominantly north-bound inshore currents typically experienced along the west coast of South Africa (Harris 1978), it is surprising that larvae have not spread to sheltered sites such as Saldanha Bay. This current pattern is maintained throughout the year (Harris 1978), with onshore currents a rare occurrence within the crab's range. While in North America, it has been shown that currents limit the spread of *C. maenas* (Behrens Yamada et al. 2015), it was inferred that a coastal upwelling may be preventing the northward spread of the crab in South Africa (Behrens Yamada et al. 2017). However, it is unlikely that this force acts in the South African context as there is no dominant upwelling centre between Table Bay and Saldanha Bay (Shannon and Nelson 1996). It is likely, however, that there are other factors determining the crabs' range, for example, predator-driven biotic resistance may be absent in the harbours (see Chapter 4 for further detail), but present in undisturbed natural systems along the coast. Such factors may also determine the distribution of different life history stages of the crab. In the native range, size classes of *C. maenas* occupy different tidal zones, with young juveniles commonly found in the intertidal habitat (Ropes 1988, Moksnes et al. 1998) while older juveniles and adults are found in the lower intertidal to subtidal regions (Crothers 1967). In its native range, this segregation is thought to reduce

competition between size classes and also offer some protection to smaller individuals who may be susceptible to cannibalism (Moksnes et al. 1998). Unlike populations of *Carcinus* elsewhere in the world, South African populations have not demonstrated this spatial segregation in natural habitats and are precluded from doing so in harbours as intertidal zones are restricted to vertical wharf surfaces.

Temporal patterns in reproductive status and abundance of the various life stages of *Carcinus* have not previously been assessed in South Africa. Observations early in the South African invasion indicated that *C. maenas* bred from July to November (Le Roux et al. 1990). This study confirmed this general pattern, with gravid females recorded from June to January. In its native range, the reproductive season of *C. maenas* is dependent on water temperature (Berrill 1982). In the crab's native northern North Sea range, where winter water temperatures drop below the minimum requirement for moulting and thus breeding, the reproductive season is restricted to a few warmer months of the year (Rasmussen 1973, Dries & Adelung 1982, Berrill 1982, Moksnes 1999). In contrast, gravid females are seen throughout the year in the southern North Sea, where mild winters are common (Crothers 1967). Along the South African west coast, coastal upwelling from January to March results in reduced water temperatures during this time (Smit et al. 2013). This frequently results in a drop below the minimum temperature requirements reported for the reproduction, brooding and growth of *C. maenas* (Berrill 1982, Behrens Yamada 2001). This may explain the absence of gravid females captured in traps between February and May, and supports the hypothesis that breeding within this species would be restricted during periods of cool water temperatures. The shortest reported generation time recorded for *C. maenas* is one year, observed in Portugal and on the Pacific coast of the USA (Queiroga 1998, Behrens Yamada & Hunt 2000). Based on seasonal similarities in water temperatures between these regions and the South African west coast (Hill et al. 1998), it was expected that the Table Bay population would exhibit a similar generation time, with a peak in gravid females coinciding with high catches of juveniles. This pattern was not observed, however, although further monitoring of the population could provide more insights. In addition, small size classes and reproductive females may be under-represented in this dataset as during the moulting phase, crabs cease feeding (Behrens Yamada 2001). Juveniles moult with a higher frequency than adults, in order to obtain significant growth in a short period of time and female crabs moult prior to mating (Behrens Yamada 2001).

Unexpectedly, no postlarval settlement was detected in Table Bay harbour over the course of a year, however, this does not indicate an absence of reproduction. Postlarvae settlement collectors, similar to those used in the current study, have been successfully used to collect *C. maenas* postlarvae in Sweden (Moksnes et al. 2003). However, due to a short reproductive season in Sweden, postlarval settlement occurs over a short period of time, specifically between June and October (Moksnes 1999). Given that gravid females are found throughout the year in South Africa (Chapter 3), it is likely that settlement also occurs

throughout the year thus making it more challenging to detect postlarvae when densities are lower. Alternatively, the sites used in this study might be unsuitable for postlarval monitoring, or the survey timing might simply have coincided with poor recruitment years. Although monitoring of the early life stages of an invasive species has considerable benefits in terms of control success following an early detection (Bax et al. 2002, Simberloff 2003, Anderson 2005, Vander Zanden et al. 2010, Beric & MacIsaac 2015), this particular methodology does not appear to be useful as a monitoring tool for this species under South African conditions.

If management of *Carcinus* is deemed to be feasible in South Africa (see Chapter 3 for further detail), this study demonstrates that control efforts should focus on the harbour populations. There is no optimal season to initiate management action, as the crab breeds throughout the year in South Africa. Despite an absence of *Carcinus* in other areas along the coast, it is recommended that monitoring for this species should continue on an annual basis. Monitoring should be focussed on sensitive areas such as Langebaan Lagoon in the West Coast National Park and sheltered sites within the Table Mountain National Park, for example, Oudekraal and Seaforth. If the species is detected early in its invasion of these sites, control efforts are more likely to be successful, thus potentially reducing the threat posed to vulnerable habitat.

CHAPTER 3: MANAGEMENT OF A MARINE INVASIVE SPECIES: INSIGHTS FROM A *CARCINUS* CONTROL PROGRAMME

Abstract

As a result of ecological impacts elsewhere in the world, *Carcinus maenas* was identified as a management priority in South Africa. This study took the methods applied in other marine management programmes together with techniques utilised in commercial fisheries, testing and adapting these in the context of the South African *Carcinus* invasion. A small population was targeted for control with the aim of assessing the feasibility of national eradication. Potential control methods (baited traps, crab condos, diver collections and sediment dredging) were trialled prior to the initiation of a pilot management programme in the semi-enclosed Hout Bay harbour. Baited traps were found to be the most practical method and were thus deployed from June 2014 to June 2015. The population was then monitored once a month for six months to track the response to management. A total of 36,244 crabs were collected during the management phase. This was six times more than a 2013 population estimate, but the population was still not extirpated. The data collected were then used to build a Bayesian depletion model to estimate the response of the population to various management scenarios. The model estimated that at the termination of management, approximately 984 individuals remained. The monitoring phase recorded an increase in catch per unit effort (CPUE) as the population recovered to an estimated 6,506 individuals. The most effective management scenario predicted by the model required a considerable increase in trapping effort (from 3,250 to 8,000 hours of effort per month) that would, after 18 months, have resulted in an estimated 86% probability of extirpation. If management was undertaken at this level of effort on a national basis (i.e. targeting Hout Bay and Table Bay), it would cost approximately ZAR 180 million. This study documents the first attempt at controlling a marine invasive species in Africa, providing administrative and scientific insights for future management programmes. Some of the key lessons learnt here illustrate the challenges in securing finances for the programme duration, the importance of targeting all life stages, and the use of depletion models in invasion management, especially if such models can be populated with data collected while management is underway. Such models can provide real-time updates on population estimates, allowing for adaptive management and improving the likelihood of a successful outcome. It is recommended that eradication of *Carcinus* from South Africa is not attempted as the population in Table Bay is considerably larger, total costs would be high, re-invasion cannot be prevented, and there is a lack of clear evidence of impacts of the species on local, natural ecosystems.

Introduction

In situations where the impacts associated with an invasive species are severe (Frazer et al. 2012) or the species is known to be problematic elsewhere (Anderson 2005), management might be deemed necessary. Meta-analyses of eradication attempts of invasive alien species can, with the benefit of hindsight, identify the prerequisites of successful management approaches (Pleuss et al. 2012, Panetta 2015). The feasibility of an eradication attempt can be assessed on two levels: 1) scientific and 2) administrative (Panetta 2009). The extent of the invasion, the biology of the target species, and the availability of appropriate control methods can determine scientific feasibility. In contrast, administrative feasibility deals with the logistics of invasive species management, with a focus on programme duration, funding, permitting, legislation, and employment contracts. Assessing scientific feasibility is often the initial step of management programmes, in particular, determining the susceptibility of the target species to the control techniques (Panetta 2015). This can be determined using previous experience where available (for example, identifying methods used on the species elsewhere) and through field trials undertaken in the local context. This is a crucial step that should be taken prior to the initiation of a large-scale eradication programme, to enable the optimal utilisation of limited resources.

As discussed in Chapter 4, *Carcinus maenas* has ecological impacts in several invaded regions globally (Grosholz et al. 2000, Mattheson et al. 2016). As a result, there has been considerable focus on management options for this species (Walton 2000, Thresher et al. 2000, De Rivera et al. 2007, Aquenal 2008, Best et al. 2014, Turner et al. 2016). These include biological, chemical and physical control, as well as novel techniques such as genetic manipulation. It has been suggested that the two most effective approaches to manage *C. maenas* are biological control and physical removal (Thresher 1997). However, developing safe and effective biological control trials in the marine environment is extremely challenging. As such, this management option has not been fully explored to date. Physical removal of these predatory crabs with traps containing non-poison bait has been the preferred method of control, especially in relatively enclosed areas such as harbours (Walton 2000, De Rivera et al. 2007, Aquenal 2008, Duncombe & Therriault 2017). However, the success of different management options can be context dependent (Shea et al. 2005), and often the most appropriate management approaches require a combination of several different techniques (Courchamp and Sugihara 1999, Genovesi 2007). This is an especially useful approach as population structure, demographics or distribution changes through time (Taylor & Hastings 2004, Chadès et al. 2011).

The aims of this chapter were firstly to identify the best management practices for *Carcinus* in South Africa and secondly, to assess the resources required to extirpate a

small population, thereby estimating the feasibility of eradicating the species from South Africa. Ultimately, the insights gained from this study will contribute to the knowledge base supporting the management of marine invasive species, both nationally and internationally.

Methods

As discussed in Chapter 2, the distribution of *Carcinus* in South Africa is currently confined to Table Bay and Hout Bay harbours. Table Bay is an official Port of Entry for the country (DHA 2014) and as a result, receives large volumes of international shipping traffic, including commercial ships, fishing vessels and yachts. This harbour supports the largest and highest density subtidal population of *Carcinus* in the country (Jooste 2013). In contrast, Hout Bay harbour is a small semi-enclosed harbour, 30 km south of Table Bay harbour (Figure 3.1). The main industries in Hout Bay harbour include tourism, recreational boating and fishing related activities (the mooring and repair of fishing vessels and fish processing factories). According to a mark-recapture survey conducted in Hout Bay in 2013, the harbour supported an estimated *Carcinus* population of 6,656 individuals (95% CI: 717 – 12,595) (Jooste 2013). Due to the smaller population present in Hout Bay harbour this location was selected to pilot a management programme targeting *Carcinus.* The data collected during the pilot programme was used to estimate the potential for national eradication (i.e. complete removal from both Hout Bay and Table Bay harbours).

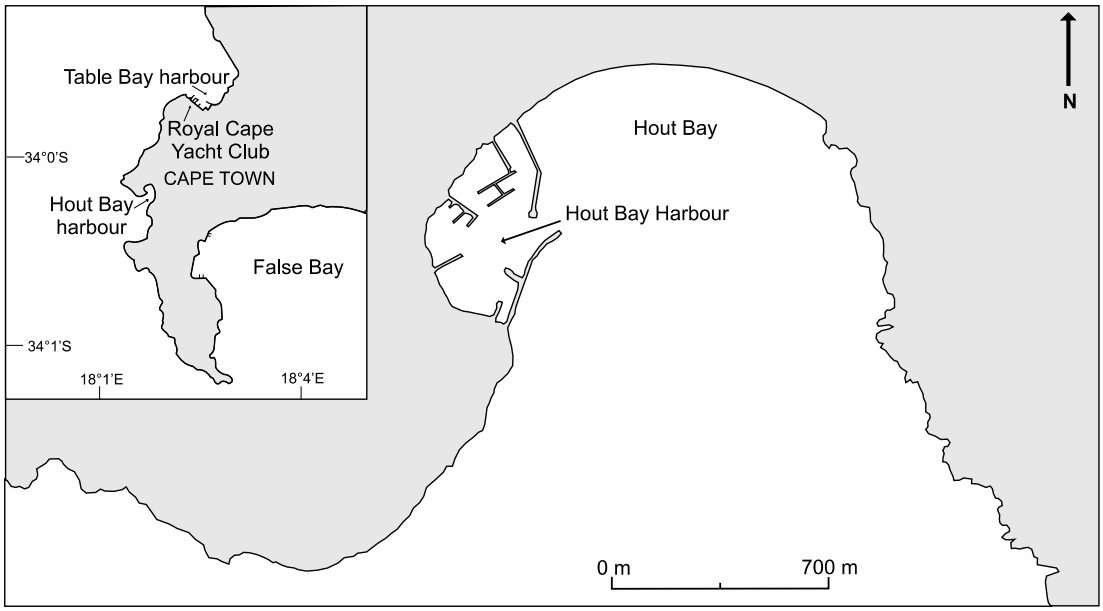

Figure 3.1. Map showing the layout of Hout Bay harbour with an inset indicating the position of the harbour on the Cape peninsula relative to Table Bay harbour.

Management plan

The original management plan for this study was developed using the previous population estimates for Hout Bay by Jooste (2013) as a guide. It consisted of three phases. The first phase involved the testing of various removal methods used elsewhere (Walton 2000, De Rivera et al. 2007, Aquenal 2008, Duncombe & Therriault 2017). The second phase planned to deploy traps targeting both juvenile and adult crabs. The target was to reach a daily catch of equivalent to or less than 10% of the maximum recorded daily catch or catch per unit effort, whereby CPUE was calculated as the trap catch divided by trap soak time (in hours). The third phase included subtidal collections by scientific SCUBA divers while deployment of traps from the surface continued. Four divers would be deployed simultaneously to collect crabs following a 50 m line in a grid pattern. It was anticipated that this would be particularly useful in the open areas of the harbour (with no surface platform) where trapping was logistically impossible due to the commercial nature of the harbour. It was anticipated that the programme would cease once no crabs were detected for 40 continuous days. This period was chosen because it corresponded to the larval period for *C. maenas* under laboratory conditions (Williams 1967, Mohamedeen & Hartnoll 1989). Thereafter monitoring of the population with traps and divers would be reduced to one day every two weeks for the following 12 months.

Trial of methods

Four methods for removing crabs were trialled. Both baited traps and crab condos were considered because juveniles can be excluded from baited traps due to competition, cannibalism or predation by larger individuals (Miller 1978, Miller 1980), whereas condos only allow smaller crabs to enter. Baited traps rely on the predatory nature of crabs, while crab condos offer a source of shelter for juveniles. The use of divers was considered for targeting crabs not attracted to the traps (i.e. those uninterested in feeding, trap-shy, or occurring in areas that were out of the range of the traps or condos). As *C. maenas* is known to bury into the sediment (Behrens Yamada 2001, Mouritsen & Jensen 2006), sediment dredging offered an approach to target individuals that were not visible to the divers. The efficacy of the various methodologies was assessed so as to identify the most appropriate for management in the South African context.

Baited traps

To establish the optimal deployment period, the CPUE of baited traps was assessed under various soak durations in Hout Bay harbour (see Figure 2.4 for further details

on trap design). Three traps were deployed at three locations for each of one, two and four hours (n = 9 per soak duration). *Carcinus maenas* trapping programmes typically have longer (i.e. 18 to 24 hour) soak times (De Rivera et al. 2007, Beal 2014, Duncombe & Therriault 2017). However, due to high crime rates in this harbour, the traps could not be left unattended and thus a shorter soak was necessary. Traps were deployed with 200 g of crushed sardines wrapped in nylon material to serve as bait. Traps were placed at least 20 m apart, at a water depth of 6 to 8 m. All trials were conducted during daylight hours in a soft-sediment benthic habitat. The CPUE was calculated as the number of crabs collected per trap per hour. As the count data were not normally distributed (with several zero counts), a Kruskal-Wallis test was used to analyse the effect of soak duration on CPUE. As with all statistics and population modelling in this chapter, the analysis was conducted in the R statistical environment (R core team, 2016).

The efficacy of baited traps was assessed under various crab densities in an outside laboratory. Crabs were collected from the Royal Cape Yacht Club and allowed a minimum of 12 hours of acclimatisation to ambient conditions prior to experiments. Trials were conducted in a 3000 l circular tank (diameter 2.75 m, height 0.75 m) with water temperatures reflecting fluctuations in ambient temperature. An equal number of crabs from the medium and large size classes (41-60 and 61-80 mm carapace width respectively) were used at four densities (12, 24, 48 and 96 crabs per trial). Small (≤40 mm) and extra-large (≥81 mm) crabs could unfortunately not be used as it was not possible to collect enough individuals of these sizes in the field. As in field trials, experiments began with the deployment of a baited trap in the centre of the tank. After two hours (the optimal soak duration determined in soak trials, see Results for further details), the size of crabs captured in the trap was recorded. The data were analysed using a Kruskal-Wallis test to assess the effect of crab density on the proportion of crabs caught per soak.

Crab condos

Crab condos (see Figure 2.3 for further details on trap design) were used to target juveniles. Condos were soaked for each of 12 hours during daylight, 12 hours during darkness, 24 hours and 48 hours as well as two bait conditions (baited and unbaited) each with four replicates. Longer soaks were necessary for these traps as those without bait required adequate time to be discovered. In order to avoid the theft of traps, this method was trialled in a secure area of the harbour. Baited traps contained 75 g of chopped sardines wrapped in nylon stockings. The CPUE was examined with respect to bait condition (two levels: baited and unbaited) and soak duration (four levels: 12 hours during daylight, 12 hours during darkness, 24 hours

and 48 hours) in a two-factor generalised linear model (GLM), using a quassipoisson error distribution.

Diver collections

In order to determine the efficacy of diver collections as a potential management method (as in other studies considering alien subtidal organisms (Anderson et al. 2005)), empty *Carcinus* carapaces were placed along a 50 m transect line, dorsal surface facing upright. Carapaces were weighed down with 10 g weights attached to the ventral surface with epoxy putty. This surface was marked with an identity code so that the details of each carapace could be accurately recorded. Each transect had a random combination of nine carapaces from each of the four size classes (small, medium, large and extra-large) placed randomly up to 1 m either side of the transect line. Three scientific SCUBA divers conducted three transects each. Empty carapaces returned to the surface were recorded as "detected" and any remaining carapaces were considered "undetected". Efficacy of each dive was calculated as the proportion of detected carapaces returned by each diver relative to the total number of carapaces on the transect line.

Sediment dredging

The presence of *Carcinus* in the sediment in Hout Bay harbour was assessed by conducting five replicate transects with a sediment dredge (Figure 3.2). The dredge was towed along the seafloor by a boat, and steered by a diver to ensure sufficient sediment collection (to a maximum depth of 0.3 m). Each transect was 0.58 m wide and 100 m long. Sediment was sieved through a 0.5 cm mesh layer at the base of the dredge and the remaining substrate was hand searched for *Carcinus*. Sex and carapace width was recorded for all individuals collected in the dredge. The number of crabs captured was examined with respect to sex (two levels: male and female) and size (four levels: small, medium, large and extra-large) using a two-factor GLM, using a quasipoisson error distribution.

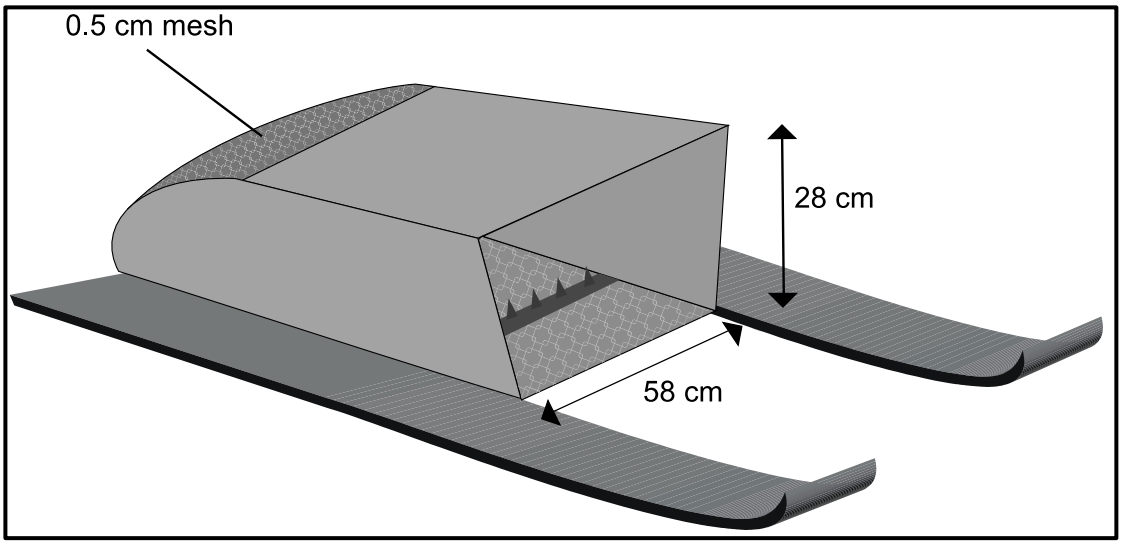

Figure 3.2. Sediment dredge used to conduct transects across the seafloor as a trial method of crab collection.

Trial Management: removing Carcinus *in a semi-enclosed harbour*

An intensive management programme was conducted in Hout Bay harbour from 23 June 2014 to 30 June 2015 (a total of 219 trapping days). The team consisted of ten field assistants and one field manager who were employed on a full-time basis. There was a management hiatus of 1.5 months during December and January due to administrative restrictions related to the reissuing of the collection permit by regulatory authorities and the appointment a new field team.

A total of 100 traps were deployed across the harbour daily, for two hours each, approximately 20 m apart on a soft-sediment benthic substrate. Each trap was baited with 150-200 g of previously frozen, crushed sardines. Crabs that were caught were sexed and sized based on carapace width. In addition, bycatch was recorded prior to release (see Appendix 3.1 for further details). At the end of the day, all crabs caught were euthanised via freezing and then incinerated as biological waste.

Following termination of the management programme in June 2015, monitoring continued for six months (July to December 2015) with 40 baited traps deployed for two hours each, once a month. This resulted in the monitoring effort reflecting 40% of daily effort during the active management phase. The methodology employed was consistent with the removal methods used during active management, except all crabs caught were released at the end of each monitoring day, to ensure that the number captured the following month was not biased by their removal. Monitoring focused on the sites identified as the most productive and accordingly targeted towards the end of the management phase. Monitoring was undertaken by a small team of scientists (comprised of volunteers and paid assistants). These sites were

deemed optimal habitat for the crabs due to the dumping of fisheries discards which would provide an easy food source.

The management and monitoring data were analysed independently of each other as they represent different approaches. Data collected during the first six days of the management were excluded from all analyses and graphs (excluding those involving cumulative catch data) as this period was considered a learning period for the field team. Catch per unit effort (defined as the number of crabs caught per eight-hour day) was examined with respect to size (four levels: small, medium, large and extra-large), month (12 levels for the management phase: July 2014 - June 2015; 6 levels for the monitoring phase: July – December 2015) and sex (two levels: male and female) using a three-factor GLM, with a quasipoisson error distribution. Pairwise t-tests with Bonferroni corrections were used on a post hoc basis. A one factor GLM with a binomial distribution was used to analyse the effect of month on the number of gravid and non-gravid females caught. Month was coded as a factor for all analyses.

Modelling predictions of population response to various management scenarios

It was apparent from the first month of management, that the previous population estimate of 6,656 (95% CI: 717 – 12,595) crabs was a gross underestimate of the current population size. Therefore, in order to obtain a more accurate estimate of the population size, data collected during the management phase were used to construct a depletion model. This model was used to predict the population size throughout management. In addition, it was used to estimate the population's response under various scenarios of on-going management in place of the management hiatus and monitoring period. Depletion models are commonly used in fisheries science to estimate stock abundance under various fishing scenarios of catch and effort (Leslie & Davis 1939, De Lury 1947). The De Lury method estimates the population size by regressing the natural logarithm of CPUE against cumulative fishing effort (De Lury 1947). The method calculates the abundance at discrete time steps that are dependent on the abundance recorded at the previous time step, the cumulative effort over this time period and the natural mortality rate (Rosenberg et al. 1990). A Bayesian State Space framework was applied to the depletion model to account for both observational and process errors and produce predictions using probabilistic rationale (Robert et al. 2010). The various size classes were combined to reduce the model complexity and CPUE data analysed using a monthly time step. While the effort data for the first six days of the management were excluded from the monthly analysis, separate analyses of catch and effort from these days were included in the model to allow for accurate predictions of population sizes. A GLM was utilised to predict the data collected under the scenarios of on-going

management action. This output was then used in a Generalised Additive Mixed Model (GAMM). The full GAMM included a smoother function (continuous spline), random effect and spatial autocorrelation for the time variable (month) while the soak time was log transformed. In order to predict crab abundance and the effort required to eradicate the population, the mean monthly CPUE (and standard error) were calculated from the GAMM. These data, along with catch and effort data, were then used as input into the state space framework.

The natural monthly mortality rate was estimated with an empirical equation commonly used in fisheries biology, including the commercially fished blue crab, *Callinectes sapides* (Hoenig 1983, Hewitt & Hoening 2005). This equation assumes an age-independent rate of mortality, as there is no published data on natural mortality rates for different age groups of *C. maenas*.

$$Monthly\ mortality = \frac{\exp\left(1.44 - 0.982 * \log(max.age)\right)}{12}$$

where $max.age$ was based on the average lifespan of four years for *C. maenas* (Behrens Yamada 2001). The natural mortality rate was considered to be constant over the course of the management programme (i.e. density independent). The model assumed that recruitment (defined here as crabs surviving from larvae to a catchable size, i.e. crabs that were too large to escape through the mesh covering the traps) was the main driver for population fluctuations. Recruitment was independent of the population size in previous time steps as it is likely that the lag time before the crabs are of sufficient size to be successfully contained within the trap is approximately 12 months (Behrens Yamada 2001). As this time frame is similar to the length of the management programme, it is assumed that all the crabs caught in the field hatched before management started, minimal recruitment is observed in the population during management and that the spawning events which produced the incoming recruits occurred prior to the start of the time series.

Bayesian State Space (BSS) modelling was conducted in Just Another Gibbs Sampler (JAGS) (Plummer 2003). This analysis used three sequential Markov Chain Monte Carlo (MCMC) simulations with 100,000 iterations each. Every tenth step was saved and the first 25,000 iterations were rejected as burn-in. The base state model predicted population size under six management scenarios (Table 3.1). In order to simulate the different scenarios, the effort level employed during management was manipulated. The mean effort level of 3,250 hours used in scenarios 2 and 3 was calculated using the data from the full months of management (July to November 2014 and February to June 2015). An effort level was simulated for scenarios 4 and 5 to depict a medium and high increase in management effort respectively. The modelling analyses used the 'lubridate' (Grolemund & Wickham 2011), 'mgcv'

(Wood 2003, 2004, 2011, 2016), ‘rjags’ (Plummer 2016), ‘R2jags’ (Su & Yajima 2015) and ‘coda’ (Plummer et al. 2006) packages in R (R core team, 2016).

Table 3.1. Details of the six scenarios used in the depletion model to predict the response of the Hout Bay population to the various management strategies.

Scenario	Description	Management time frame	Management effort used in model	Monitoring time frame	Management hiatus
S0	No management/monitoring	N/A	N/A	N/A	N/A
S1	Baseline: modelling what actually took place	**10.5 months:** Jul '14–Dec '14 Jan '15–Jun '15	Realised effort Realised effort	**6 months**: Jul '15–Dec '15	**1.5 months:** Dec '14–Jan '15
S2	As S1, but management employed instead of monitoring	**16.5 months:** Jul '14–Dec '14 Jan '15–Jun '15 Jul '15-Dec '15	Realised effort Realised effort Simulated effort: 3,250 hours	N/A	**1.5 months:** Dec '14–Jan '15
S3	As S2, but no management hiatus	**18 months:** Jul '14–Nov '14 Dec '14–Dec '15	Realised effort Simulated effort: 3,250 hours	N/A	N/A
S4	Continuous management (moderate increase in effort)	**18 months:** Jul '14–Dec '15	Simulated effort: 5,000 hours	N/A	N/A
S5	Continuous management (large increase in effort)	**18 months:** Jul '14–Dec '15	Simulated effort: 8,000 hours	N/A	N/A

Administrative costs

Reports of actual management costs for invasive species are often excluded from the scientific literature. However, this information is extremely useful for future management plans and also to analyse the total costs associated with invasive species. The South African National Biodiversity Institute (SANBI) employed all personnel involved in the management programme of the current study on a contractual basis. Salaries ranged from ZAR 32.34 – 67.93 per hour based on educational background and government-defined pay rates. The bursary costs for the lead scientist during the 12 months of management was included in lieu of a salary. Equipment was purchased prior to management so represented a once-off cost (apart from the occasional replacement or repair cost). The bait used daily during the management and monitoring phases was a regular expenditure. Storage costs included the rental of the space in which the freezer was stored and electricity charges associated with this over the period of 18 months. Expenses were incurred in transporting the crabs to the disposal facility as well as the driving costs for the principal researcher and the team involved during monitoring. Disposal fees (for the incineration of the euthanised crabs) were paid by the Biodiversity Management Branch of the City of Cape Town but are included in the total costs incurred by South Africa. The costs of conducting the management trials are not included.

Results

Trial of methods

Baited traps

There was no significant difference in CPUE between the various soak durations for baited traps ($H = 3.015$, $df = 2$, $p = 0.22$, Figure 3.3a). It is however notable that variability declined with increasing soak time. Despite the lack of significant statistical differences among soak times, there was a trend of declining CPUE with increasing soak duration. As such, an intermediate soak duration of two hours was selected for implementation so as to minimise the logistical constraints associated with multiple one-hour soak periods while maximising the yield of crabs. Notably, crab density did not affect the proportion of crabs caught during the efficacy trials ($H = 1.317$, $df = 3$, $p = 0.73$, Figure 3.3b).

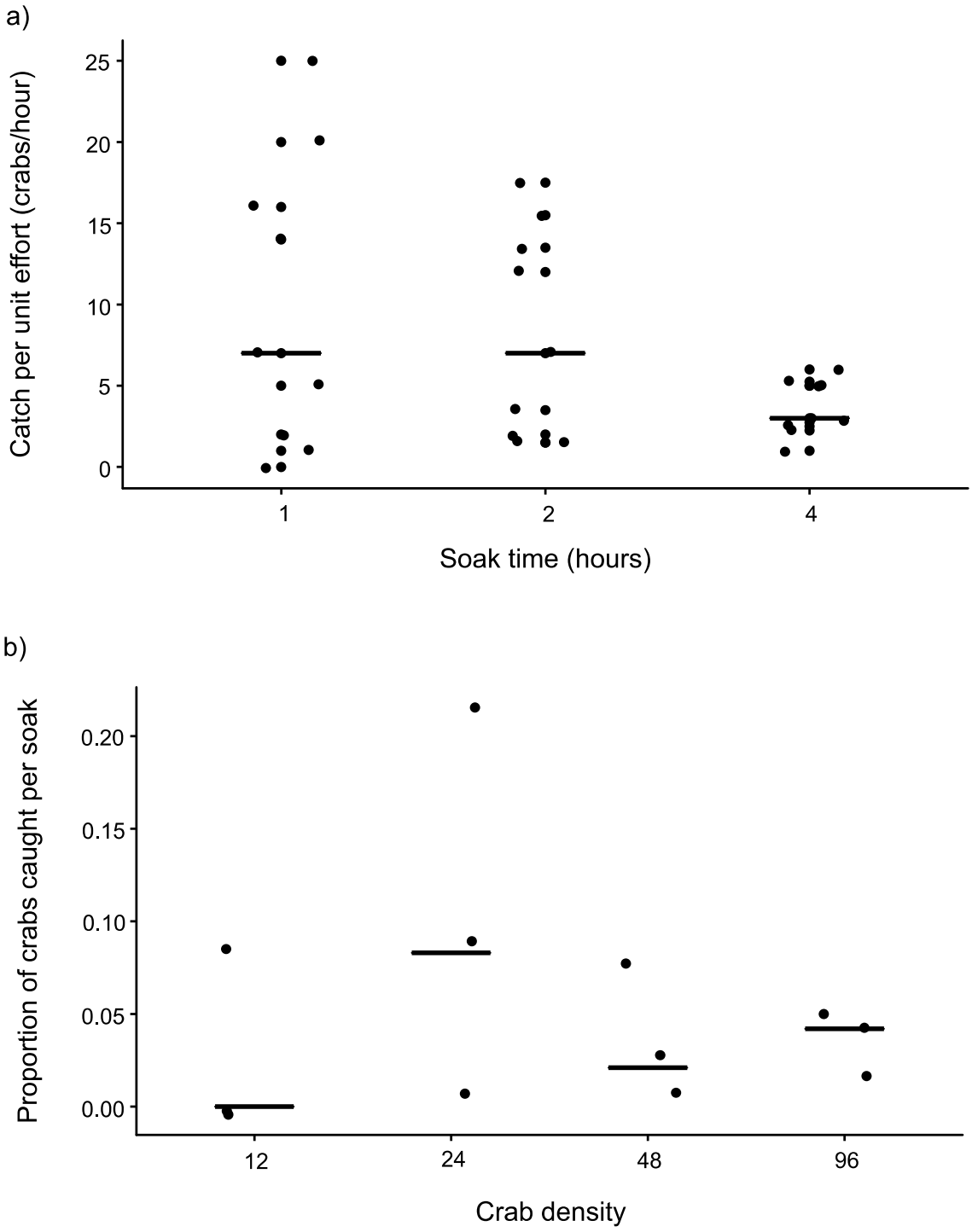

Figure 3.3.a) Jitterplot showing median catch per unit effort (CPUE) (horizontal line) standardised as the number of crabs caught per hour of trapping and raw data for the baited traps for each soak duration (1, 2 and 4-hour soak times); b) Jitterplot showing the raw data and the median proportion of crabs caught per 2-hour soak during the baited trap efficacy trial for the various crab densities (12, 24, 48 and 96 crabs in the enclosure). All differences were non-significant ($p > 0.05$).

Crab condos

In the total of 768 hours that crab condos were deployed, no *Carcinus* or native crab species were caught in Hout Bay harbour. The sardines in the baited condos remained untouched at the end of the soak.

Diver collection

Divers recovered a total of 55 crab carapaces out of 100 placed along a transect line during the efficacy survey, with a median efficacy per diver of 56% (range 27 to 83%).

Sediment dredging

All life-history stages of *Carcinus* were absent from the dredged substrate collected in Hout Bay harbour. Other macrofaunal species were retained within the dredge, but this did not include any native crabs.

Trial Management: removing Carcinus *in a semi-enclosed harbour*

Over a period of 12 months and a total of 219 trapping days, 36,244 crabs were collected in Hout Bay harbour, approximately six times the previously estimated population size and three times its upper estimate (6,656; 95% CI: 717 – 12,595) (Jooste 2013). The present study surpassed the lower 95% confidence interval of that population estimate on the eighth day, the population estimate on day 31 and the upper 95% confidence interval on day 45 (Figure 3.4). The maximum daily catch (1,079 crabs) was reached after 16 days of trapping, while the lowest catch (13 crabs) was recorded 361 days after the initiation of management.

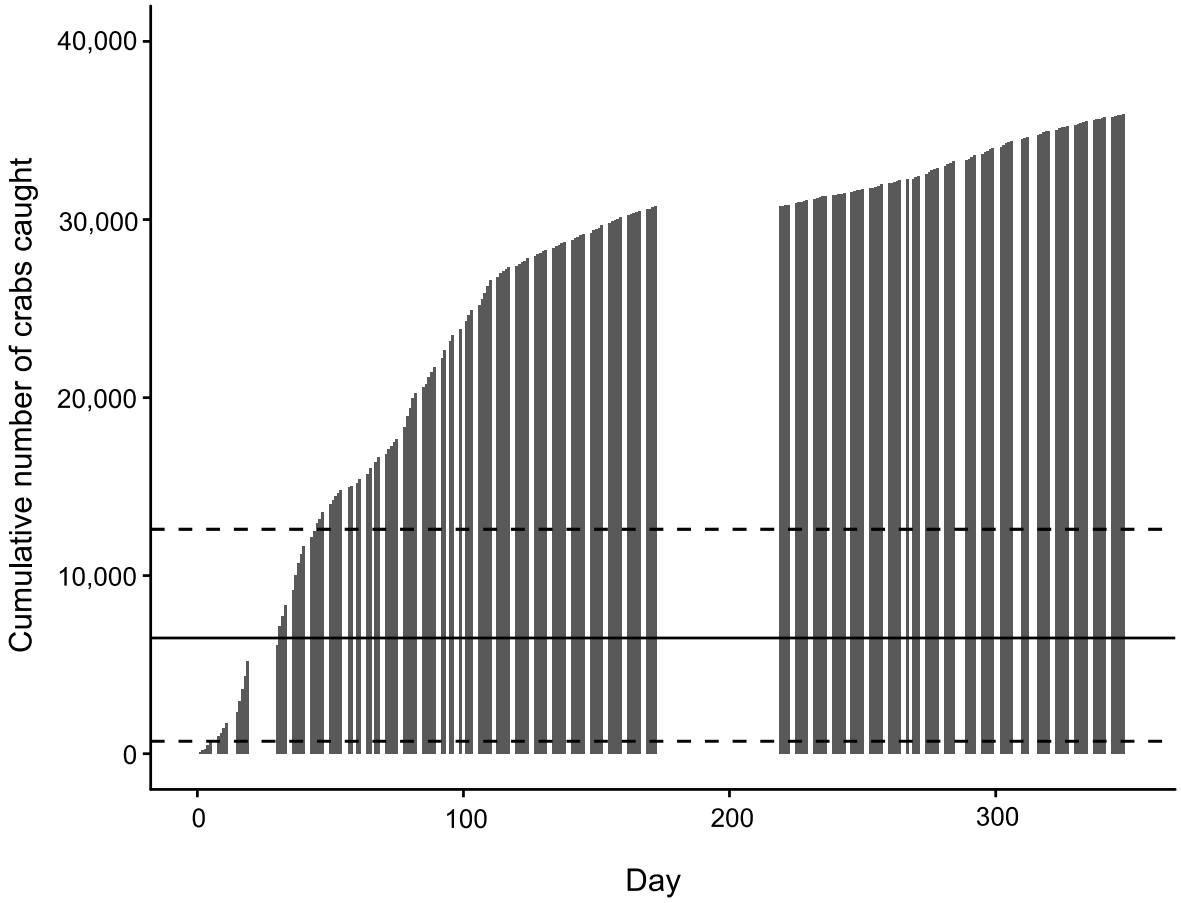

Figure 3.4. Cumulative number of crabs caught during management, from Day 1 on the 23rd June 2014 to Day 219 on 30th June 2015. Time on the x-axis includes non-trapping days such as weekends and a 46-day hiatus over December and January (due to administrative issues). The horizontal lines represent the previous population estimates (Jooste 2013): lower dashed line (lower 95% confidence interval); solid line (population estimate of 6,656) and upper dashed line (upper 95% confidence interval).

During the monitoring phase, which followed active management, the daily catches showed an increasing trend (Figure 3.5) with the highest daily catch recorded in November 2015 (five months after management was terminated) when a total of 120 *Carcinus* were collected. The catches decreased in December, which was the last month of monitoring.

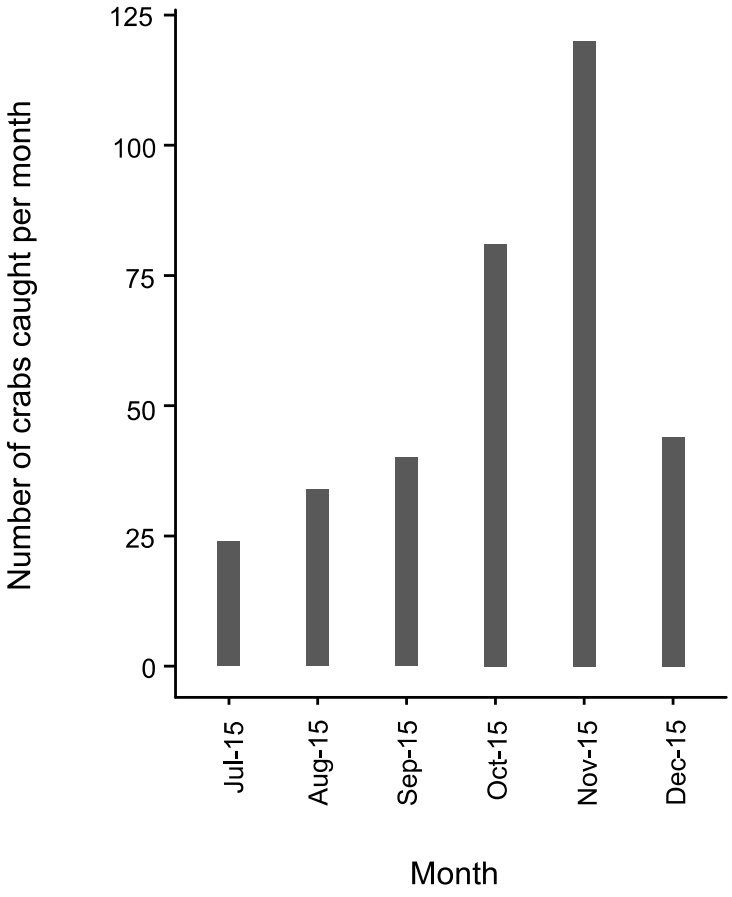

Figure 3.5. Number of crabs caught during the six-month monitoring phase (non-cumulative counts).

Size, sex and month (and the interactions between these main effects) all significantly affected CPUE (Table 3.2). CPUE was significantly different between all size classes (pairwise comparisons; $p < 0.001$) except for the small and extra-large crabs which both had low CPUE ($p = 0.76$). In the first few months of active management, the highest CPUE recorded was that of large crabs (Figure 3.6). However, the highest CPUE recorded towards the end of the management phase was that of medium (41-60 mm) crabs. The CPUE of males and females was significantly different ($p < 0.001$), with more females collected than males during the first half of the active management phase (Table 3.2). However, the sex ratio of females to males caught per day reached 1:1 as management action continued (Figure 3.7). CPUE declined significantly among months from July to November 2014 ($p < 0.001$), and thereafter remained at low levels.

Table 3.2. GLM results considering the effect of crab size category (4 levels), sex (2 levels) and month (12 levels) on catch per unit effort (CPUE) during active management. See Appendix 3.2 for further details regarding the GLM output. * indicates a significant result.

Factor	df	F	*p*
Size	3	8,745.21	<0.0001*
Sex	1	9,733.89	<0.0001*
Month	11	1,538.97	<0.0001*
Size x Sex	3	135.07	<0.0001*
Size x Month	33	67,47	<0.0001*
Sex x Month	11	115.62	<0.0001*
Size x Sex x Month	33	2.77	<0.0001*

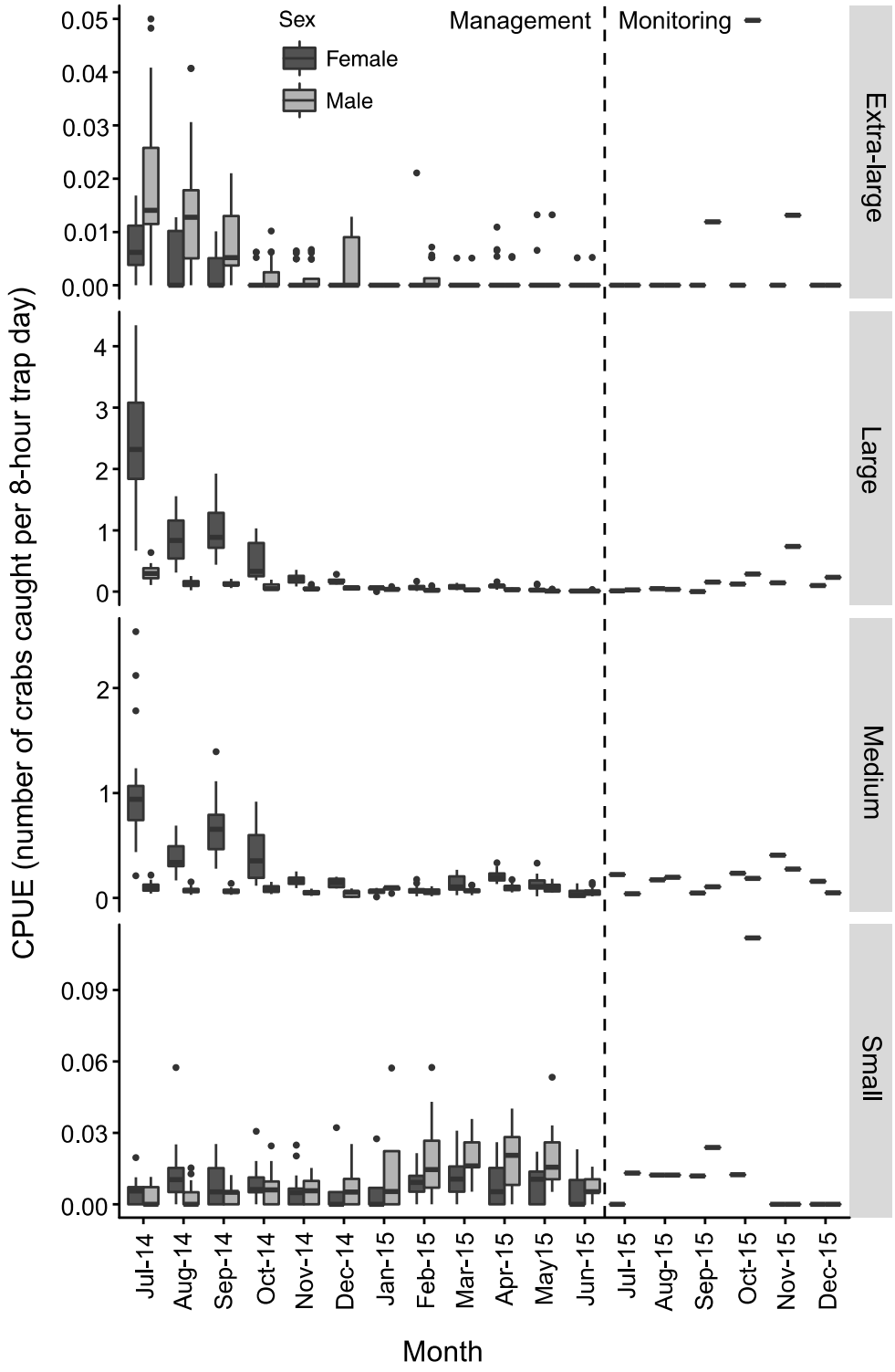

Figure 3.6. Boxplot showing the catch per unit effort (CPUE) of male and female *Carcinus* in the different size classes during the active management and monitoring phases. CPUE is measured as the number of crabs caught per eight-hour trap day. Size classes are defined based on carapace width: small ≤40 mm; medium 41-60 mm; large 61-80 mm and extra-large ≥81 mm. Note the scale differences on the y-axes.

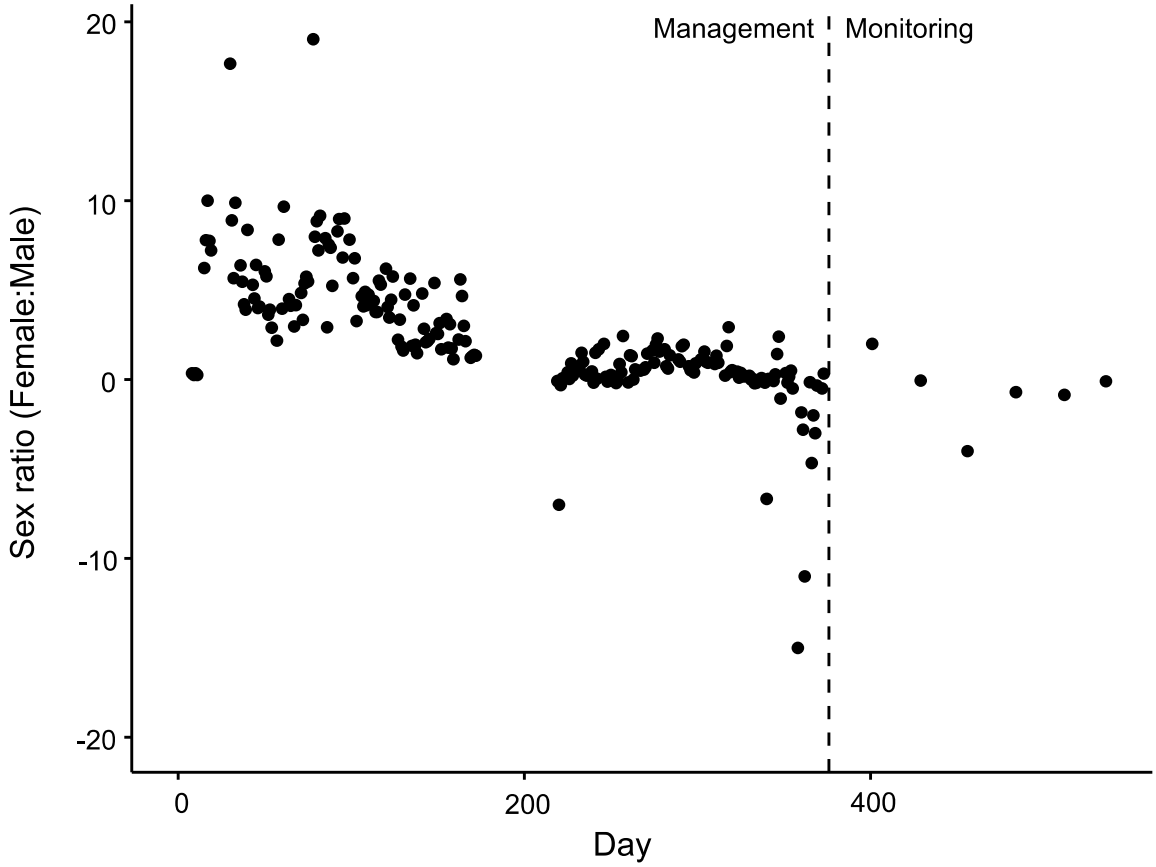

Figure 3.7. Fluctuations in the *Carcinus* sex ratio recorded per day during management and monitoring in Hout Bay harbour. Positive values indicate a female skewed sex ratio, while negative values represent male skew. A sex ratio of zero represents an equal number of males and females.

During the monitoring phase, the effects of size, sex and month were significant, as were all interactions, except the three-way interaction (Table 3.3, Figure 3.6). CPUE was lowest for extra-large and small crabs (no significant difference between these size groups (p = 1.0) while catches of medium and large crabs were higher (p = 1.0). The CPUE of males was significantly higher than females (p = 0.027). The CPUE recorded in November, the second last month of monitoring, was significantly higher than all other months (p < 0.001), except for October (p = 0.076). Of the total females captured during monitoring, 1,158 were gravid. Gravid females were found in every month of the year with no significant effect of month on the abundance collected (X^2= 288, df = 272, p = 0.242) (Figure 3.8).

Table 3.3. GLM results of catch per unit effort (CPUE) during the monitoring phase with respect to the main effects of crab size category (4 levels), sex (2 levels) and month (6 levels). See Appendix 3.3 for further details regarding the GLM output. * indicates a significant result.

Factor	df	F	*p*
Size	3	81.46	<0.0001*
Sex	1	11.46	0.0007*
Month	5	20.45	<0.0001*
Size x Sex	3	12.02	<0.0001*
Size x Month	15	3.49	<0.0001*
Sex x Month	5	3.84	0.0018*
Size x Sex x Month	15	0.76	0.7237

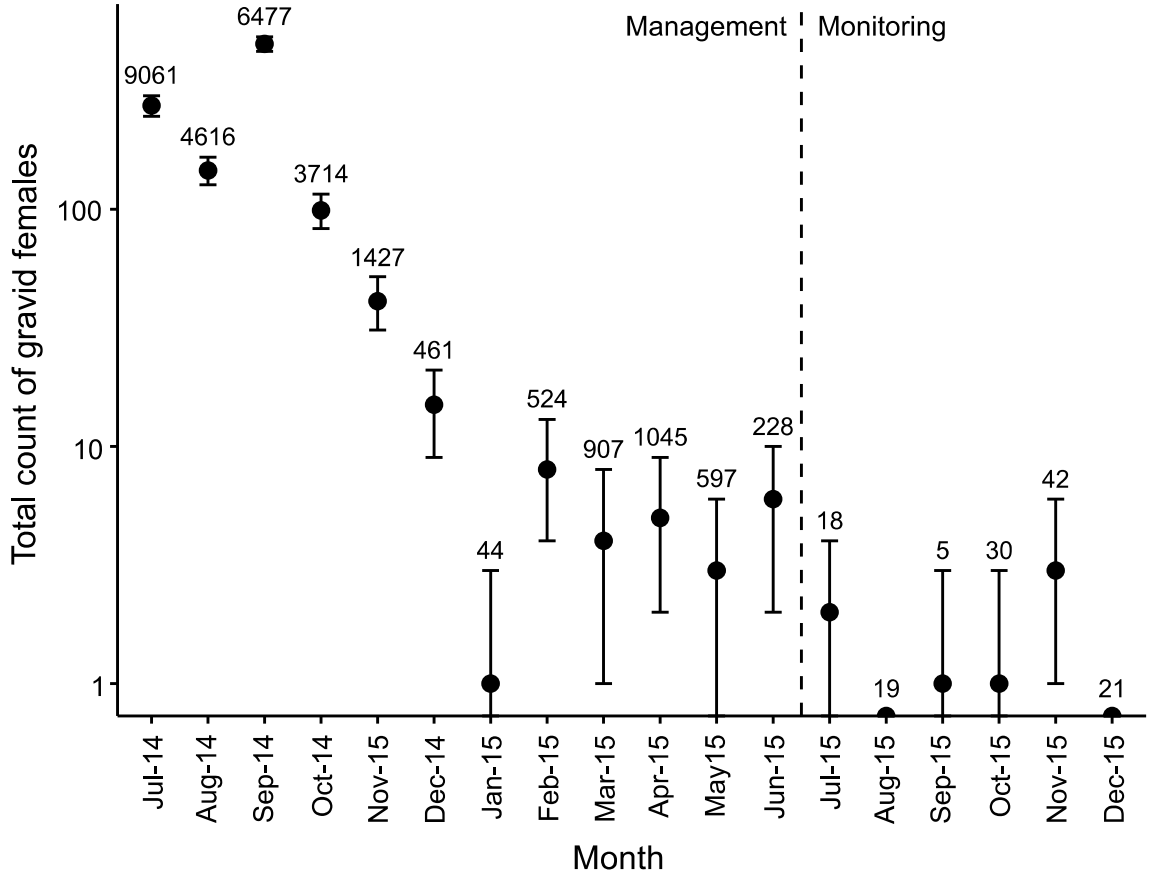

Figure 3.8. Patterns in the abundance of gravid and non-gravid female crabs captured during management and monitoring in Hout Bay harbour. The total counts of gravid females per month are summed over the total replicates and displayed with 95% confidence intervals. The total number of non-gravid females is displayed above each point. Gravid females were caught in every month.

Modelling predictions of population response to various management scenarios

The Bayesian depletion model accurately predicted the observed CPUE with an estimated initial population size of 19,624 crabs (95% CI: 16,413 – 23,355) (Figure 3.9a, b). At the end of the management phase, the population was reduced by approximately 93% and was estimated to constitute 1,482 crabs (95% CI: 530 – 2,875). During six months of monitoring, the population increased to an estimated 6,621 individuals (95% CI: 3,227 – 11,904) (Figure 3.9c). The total estimated recruitment over the 18-month period consisted of 31,830 crabs (95% CI: 26,516 – 39,332) with the highest recruitment peak observed during September 2014 (Figure 3.9d).

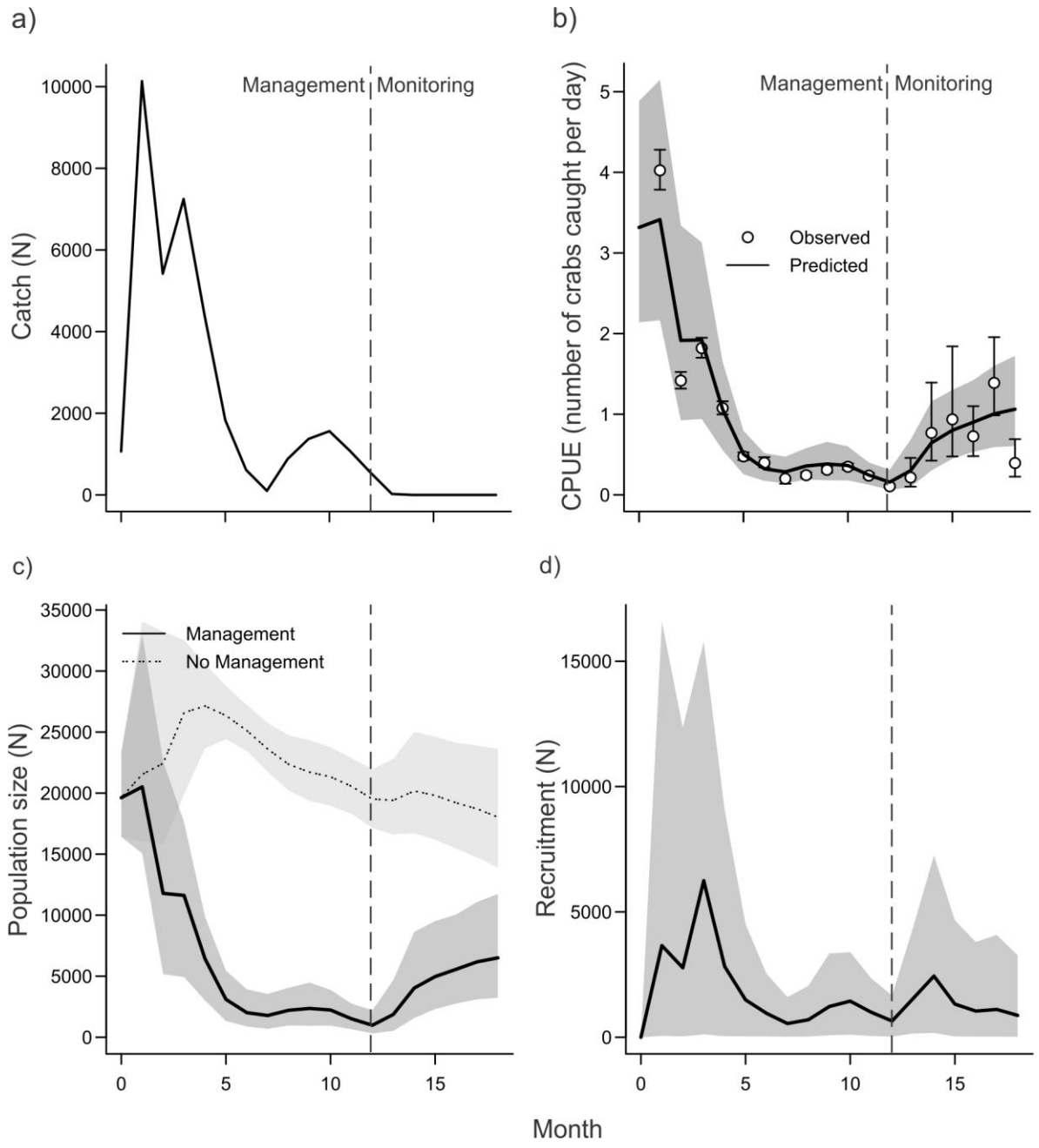

Figure 3.9. a) Catches of *Carcinus* through time during the active management and monitoring phases of the control programme; b) Observed and predicted catch per unit effort (CPUE) over the same period; c) Model predictions of the population size under two scenarios: no-management (S0) and realised management (S1); d) Recruitment of crabs into the 'catchable' population through time. In all graphs the shadowing represents the 95% confidence intervals. Month 0 is June 2014, while Month 18 is December 2015.

The model predicted that if management had continued unabated (Scenario 2), the population would have consisted of 1,701 individuals (95% CI: 201 – 4,939) at the end of 2015 (Figure 3.10a). If there had been continuous management action for 18 months with no hiatus over the December 2014 to January 2015 period (Scenario 3), it was predicted that by December 2015, the population would have consisted of 1,700 individuals (95% CI:

200 – 4,933). Increasing the effort level to 5,000 hours per month (Scenario 4) would have reduced the population to 940 crabs (95% CI: 156 – 3,649) while a further increase to 8,000 hours of effort (i.e. Scenario 5) would have been the most effective management strategy with a final population size of 153 crabs (95% CI: 0 – 1,735). Under the latter scenario, there would have been an 86% probability of extirpation (Figure 3.10b).

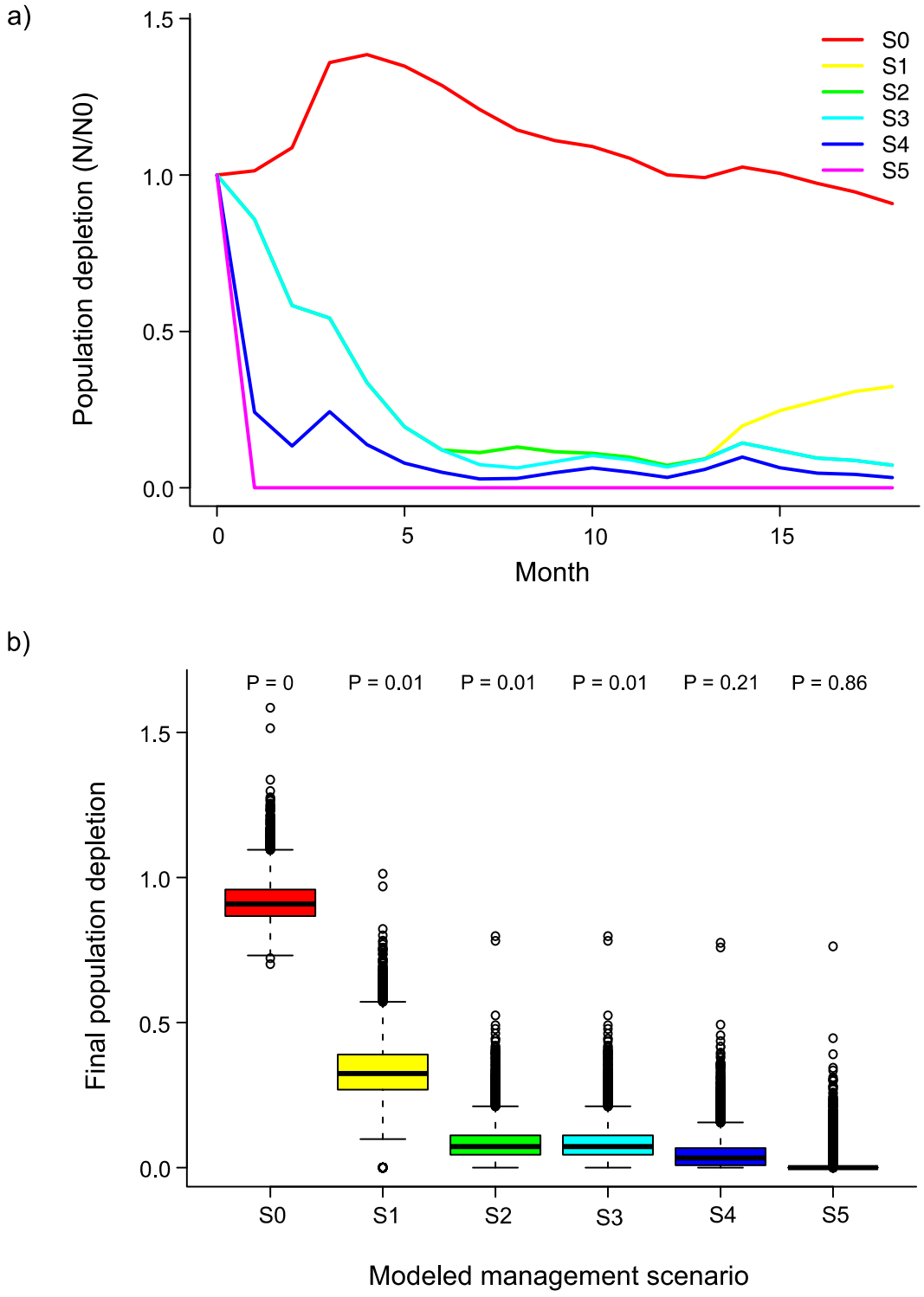

Figure 3.10. a) Scenario projections from the De Lury model depicting the depletion of the crab population in Hout Bay through time relative to the initial population size, see Table 3.1 for detailed description of the scenarios; b) The probability of extirpation (P) of the crab population relative to the final depletion under the six simulated management strategies, 75,000 iterations for each strategy.

Administrative costs

The greatest cost of the management phase was for the payment of salaries, with this amounting to ~ZAR 750,000 (Table 3.4). The total person hours worked during the active management period was 18,450 hours. Over the 218 trapping days, the mean daily expenditure on salaries was ZAR 2,786. Personnel costs were much lower during the monitoring phase, amounting to a total of ZAR 2,350 for the six days of work. However, as this management programme was co-ordinated by a PhD student, the salary for the lead scientist was low. If a similar management programme was to be undertaken in the future, there would likely be additional personnel costs for this project co-ordinator.

Table 3.4. Daily and total costs (in South African Rand (ZAR) at 2016 levels) from the active management and monitoring phases.

Category	Management daily	Management total	Monitoring daily	Monitoring total	Grand total
Personnel	3,278	734,250	392	2,350	**736,600**
Lead scientist	274	100,000	0	0	**100,000**
Equipment	32	7,201	0	0	**7,201**
Storage	37	8,269	530	3,181	**11,450**
Transport	7	1,566	65	393	**1,958**
Bait	52	11,612	17	102	**12,559**
Disposal	50	11,194	0	0	**11,194**
Total	**ZAR 3,730**	**ZAR 874,092**	**ZAR 1,004**	**ZAR 6,025**	**ZAR 884,632**

Discussion

This study represents the first effort to manage a marine invasive species on the African continent. A local management plan was developed in the South African context based on the results from a trial of possible control methods used elsewhere in the world. When this plan was unsuccessful (i.e. extirpation was not achieved), further understanding of the system became necessary and methods from fisheries science were employed. In the process, practical management skills and valuable insights (from both institutional and scientific perspectives) were gained and these can contribute to the development of future management programmes, in South Africa and internationally.

Trial of methods

The typical methods used to catch *Carcinus* internationally required some adaptation to the South African context. While the international practice is to soak traps for approximately 24 hours (De Rivera et al. 2007, Aquenal 2008, Beal 2014, Duncombe &

Therriault 2017), this approach was impractical during this study as a result of the high opportunistic crime rates which resulted in the theft of several traps, even during daylight hours. While trap saturation is commonly observed in baited crustacean traps (Miller 1978, Miller 1979, Beal 2014) with traps that are retrieved on a more regular basis reporting greater overall catches (Miller 1979), in this study, catches were not significantly affected by soak duration, at least not at the scale of one to four hours. As such, in an effort to balance the logistical challenges associated with short trap deployments while maximising catches, a management decision was made to employ a two-hour soak period for this study. Trapping effort could be increased in other ways including trapping on weekends (i.e. introducing a seven-day week with additional staff), or by increasing the number of traps deployed on a daily basis by staggering the release of extra traps during the original soaks. The latter option would require the construction of additional traps but no additional personnel costs. This would allow for an increase in effort and correspondingly the probability of extirpation while minimising costs. Under other circumstances, where crime rates are not an issue, longer trap soak durations could be trialled once the population is reduced. The ability to detect the target species at low densities (either towards the end of management or soon after its arrival) is an essential prerequisite for successful management (Myers et al. 2000) and thus it was necessary to assess the capacity of traps to achieve this. As in other studies (Miller & Addison 1995), the proportion of crabs captured in the baited traps was not affected by density. Therefore, even at low densities towards the end of management, the traps used in this study are expected to successfully target *Carcinus*.

Crab condos have been used successfully in Australia to capture small *C. maenas* (Hewitt & McDonald 2013). However, the condos (both baited and unbaited treatments) were unsuccessful at capturing *Carcinus* in Hout Bay. It was expected that the unbaited condos would be less successful than the baited condos, as other crab species are known to show limited interest in traps which contain no bait (Miller 1978). However, this does not explain the low efficacy of the baited condos. It is unlikely that this reflects an ineffective trap design as traps of the same design were successful in Table Bay harbour (see Chapter 2). It is, however, possible that the inefficacy of baited condos in Hout Bay reflects the fact that they were deployed in an area that was later found to support low densities of *Carcinus*. This was unavoidable as it was the only secure area in the harbour in which traps could be left for prolonged periods of time without a high risk of being stolen. The limited success of the condos in this study meant that they were not included in the active management phase, unavoidably resulting in a high likelihood that juveniles were under-represented in catches. It is not clear the extent to which an inability to catch juveniles would have affected the likelihood of eradication.

The diver efficacy experiment specifically assessed the ability of the divers to find crab carapaces placed on a transect, whereas in reality, the crabs would be mobile, making them easier to detect if they were to move across the diver's line of sight. Marine invasive

species have previously been targeted using volunteer SCUBA divers (e.g. the collection of lionfish in the Caribbean (Frazer et al. 2012) and zebra mussels in the USA (Wimbush et al. 2009)). However, in South Africa, all biological sampling is classified as non-recreational diving and therefore, legally all divers need to be commercially certified, working in a minimum team of four people with monetary reimbursement for their time (Occupational Health and Safety Act 2002). Due to this legislation, the use of volunteers for such management efforts would be illegal in the South African context. This has important financial implications for management authorities wishing to use divers as the cost of hiring a commercial dive team, in addition to the cost of boat hire and fuel, would be approximately ZAR 8,000 a day. Notably, only around half of crab carapaces placed during the efficacy experiment were found by the divers. Thus, due to the low efficacy of this method, and the cost implications, diver removal is not seen as a long-term management option in South Africa, particularly not for reducing large populations. It might, however, be used strategically to target areas inaccessible to traps, in an attempt to remove the last few individuals, or as a check before declaring that a population has been eradicated. They could also be employed to collect moulting or reproductive crabs that are unlikely to be interested in feeding and thus unlikely to enter baited traps (Behrens Yamada 2001).

The collection of crabs by sediment dredging was unsuccessful in this project. As the dredge was pulled behind a boat, only the open areas could be accessed. The fact that *C. maenas* commonly avoids these areas, preferring darker areas (Moksnes et al. 1998, Barr & Elwood 2011), likely explains the absence of crabs in dredged sediments. Therefore, it might be that this method of collection is not appropriate for this species, particularly in harbours. It might, however, have more applicability to species that are solely sediment dwelling, such as clams or aquatic plants (Eichler et al. 1993, Wittman et al. 2012). In addition, this method requires the use of a commercial dive team and accordingly the costs discussed above make this an expensive management tool.

The collection of postlarvae was not attempted during this study as postlarval settlement collectors were previously found to be ineffective (see Chapter 2). While plankton tows could have been used to collect larvae (DiBacco & Therriault 2015), such methods would require an understanding of the factors that determine the timing of larval release as well as the regular use of a boat in order to tow the nets. To date, no peak in larval abundance has been identified in South Africa (see Chapter 2) and thus it is likely that larvae are released at low levels throughout the year and accordingly might be below the detection level. In its native range, *C. maenas* larval abundance peaks outside of daylight hours (Queiroga et al. 1994), but sampling in Hout Bay after sunset would present a safety and security risk and thus was not attempted. Considering the above, it is clear that larvae, postlarvae and juvenile crabs were unavoidably not targeted by the methods used in this study. This is likely to have reduced the probability of management success.

Therefore, while only baited traps were used in Hout Bay, if an attempt was made to eradicate the crabs from elsewhere, a mix of strategies would be recommended. The utilisation of a combination of methods has previously been highlighted as the most appropriate approach to management (Courchamp & Sugihara 1999, Wittenberg & Cock 2005, An et al. 2007). From the experience in this study, it would be best for baited trapping to be applied on a daily basis with divers employed strategically, and crab condos used where they are found to be effective.

Management and monitoring of Carcinus

During the course of this study, the total number of crabs removed from the harbour was much higher than the most recent population size estimate (Jooste 2013). The inaccuracy of the previous estimate might have resulted from the violation of an assumption associated with the mark-recapture method employed (i.e. unequal catchability of individuals or insufficient mixing of the marked crabs back into the population between sampling occasions). Unavoidably the current model might also have violated some assumptions. The depletion and mark-recapture models assume that all individuals in the population have equal catchability, with catchability remaining constant over the sampling period (De Lury 1947). However, this assumption is rarely met when targeting decapods (Miller & Mohn 1993). Indeed, in Hout Bay, the crabs captured in the traps at the beginning of the management period were significantly larger than those recorded towards the end, possibly due to aggressive behaviours which can deter other individuals from entering the traps. In addition, breeding or moulting crabs would not be attracted to the baited traps (Behrens Yamada 2001). As unequal catchability affects both models, it is unlikely to be the cause of the discrepancy between these population estimates but it might explain the difference between the models and the actual number of crabs captured.

In addition to the assumption of equal catchability, both models assume that the Hout Bay population is closed to migration and recruitment. A *C. maenas* trapping study that attempted to deplete a population using short periods of intensive management over consecutive years, found that when management resumed that, in some years, population size has increased significantly (Duncombe & Therriault 2017). This was attributed to strong recruitment episodes during the absence of management (Duncombe & Therriault 2017). As predicted by the current model, recruitment into the 'catchable' population is the most likely cause of the discrepancy between the estimates and the realised catch. This would arise when small individuals are not retained within the trap mesh of the traps until they obtain a critical size. This effect would not be relevant over the short duration (i.e. four days) of the previous survey in Hout Bay (Jooste 2013). In the present study it is unlikely that larvae hatched during the management effort could have recruited during the project, as (based on reported growth rates (Behrens Yamada 2001)) the crabs would

still be small enough to escape through the mesh. However, juvenile crabs hatched prior to 2014, might have reached a catchable size during management, especially as the condos were ineffective at capturing the small crabs. Based on the modelling simulations, the original population consisted of 19,562 individuals prior to management action. Although abundance was considerably reduced as a result of management, it appears that high recruitment levels into the population made complete removal unlikely, with 31,325 crabs recruited over the 18-month period of management and monitoring.

It should be acknowledged that the depletion model used in the current study assumes a rate of constant mortality, in the absence of available data on *Carcinus* mortality rates. However, this assumption is likely to be violated, particularly once the larger crabs had been removed from the Hout Bay population (see below for further discussion). *Carcinus maenas* is known to be cannibalistic in its native range (Moksnes 2004, Almeida et al. 2011) and thus the effects of management in the present study are likely to alter this mortality rate through time.

During management, the expectation is that as the number of individuals detected or CPUE declines through time, the probability of extirpation increases (Panetta 2007). Active management of the Hout Bay *Carcinus* population resulted in the removal of 36,244 crabs, with a significant decrease in CPUE through time. Such decreasing catches or reduced densities are commonly observed in management programmes (Panetta 2007) and have previously been recorded by various programmes targeting *C. maenas* (De Rivera et al. 2007, Aquenal 2008, Turner et al. 2016, Duncombe & Therriault 2017). A management programme in California (undertaken within an area of approximately 4 km^2) removed 9,691 crabs over a period of 66 trapping days (De Rivera et al. 2007). Over this time, mean CPUE decreased from an initial 21.3 to 1.4 crabs per trap. A later study that involved three years of intensive management in the same bay reported a mean catch of 2.6 (SE ± 1.01) crabs per day prior to management, decreasing to 1.1 (± 0.2) towards the end (Turner et al. 2016). A similar programme in New South Wales, Australia, focused on a smaller bay (approximately 0.05 km^2) (Aquenal 2008). At the start of the project, over 200 animals were caught per night and this declined to three individuals per night nine months later (Aquenal 2008). While the CPUE of these studies is not directly comparable with the present study as the methods differ, the observed trends are similar. Surprisingly, even in the absence of management action (Scenario 0), the depletion model estimated that the crab population in Hout Bay declined. This could be due to seasonal fluctuations in abundance and/or catchability, as demonstrated in other *Carcinus* populations (Duncombe & Therriault 2017).

Although *C. maenas* trapping programmes have observed decreasing catches during the removal period (De Rivera et al. 2007, Aquenal 2008, Turner et al. 2016, Duncombe & Therriault 2017), there is nothing reported in the literature regarding the immediate response of the populations following the termination of management, a key component

to understanding how quickly crab populations may recover. The CPUE increase observed during the monitoring phase in the present study might have arisen through various scenarios, including: 1) recruitment (as discussed above); or 2) overcompensation as a result of reduced intraspecific pressures, for example, competition (Govindarajulu et al. 2005, De Roos et al. 2007, Zipkin et al. 2008). Overcompensation could occur when changes to population dynamics manifest in altered reproduction rates, juvenile survival rates or time to maturation (Govindarajulu et al. 2005, De Roos et al. 2007, Zipkin et al. 2008, Zipkin et al. 2009). No evidence for overcompensation (specifically, increased juvenile abundance) was detected in the Californian population of *C. maenas* following management (Turner et al. 2016). When adult densities were experimentally increased, adult cannibalism of the juvenile crabs was low and there were minimal effects on growth and survival rates (Turner et al. 2016). If overcompensation were to occur in Hout Bay, it could allow the population to remain stable despite depletion attempts. This effect might be 'hidden' as the new recruits initially avoid capture (or census) due to their small size. Although this is a possible driver of increased CPUE in Hout Bay following the reduced depletion pressure, this effect was not directly assessed in the present study. The decreased catches reported during the last month of monitoring follow the trend that has previously been noted in the nearby Table Bay population. In the summer months in Table Bay harbour, catches of *Carcinus* were significantly lower than other months (see Chapter 2). These low catch rates might be caused by decreased water temperatures typically experienced in the summer months in this region (Smit et al. 2013) or an increase in mating activity with crabs displaying minimal interest in feeding (Behrens Yamada 2001). However, as this decreased CPUE was observed during the last month of monitoring, there is no way of knowing whether the trend might have continued.

The majority of crabs captured during management were large, with a carapace width in the range of 61 to 80 mm. However, the median sizes of the captured crabs decreased through time. This is a common trend in crustacean fisheries, as larger individuals are often more aggressive, preventing smaller individuals from entering the traps (Miller 1978). This is undesirable in commercial fisheries as the smaller sizes are generally below the legal capture size and traps are specifically designed to reduce the capture of small individuals (Miller 1990). However, invasive species eradication programmes should ideally aim to prevent reproduction (Genovesi 2007, Panetta 2007, Pardini et al. 2008, Wilson et al. 2017). *Carcinus maenas* is capable of reproducing from a carapace width of 27 mm (Mohamedeen & Hartnoll 1989). Other *Carcinus* trapping programmes have reported similar decreases in size (De Rivera et al 2007, Beal 2014). In Canada, an average decrease in carapace width of 4% was reported at the end of the harvesting period (Beal 2014), while in California, a decrease in the size of crabs captured was followed by further increases above the initial size which the authors attributed to individual growth (De Rivera et al 2007). The size of female crabs affects the number of eggs they can carry (Behrens Yamada 2001), therefore all crabs of reproductive size should be targeted but

especially the large ones. In this study, although there were lower catches of large crabs even towards the end of the management, an increase in the number of small crabs surviving to reproductive age likely increased. Therefore it is possible that propagule pressure in the form of recruitment was maintained throughout this project. As the population structure changed during management (i.e. the majority of the large crabs were removed), the reduced competition might have resulted in a redistribution of the smaller crabs into the prime habitat patches. These are likely to be the areas where the commercial fisheries discards were dumped on a daily basis providing a regular food supply for the crabs.

Initial catches were highly skewed, with many more female than male crabs caught at the beginning of the management programme, although the ratio became more balanced through time. In contrast, in California, more males were captured than females at the beginning of management (De Rivera et al. 2007). It was suggested that this might be caused by males exhibiting aggressive behaviour and thus preventing female trap entry, or as a result of seasonal differences in behaviour (De Rivera et al. 2007). A similar trapping study in Canada found a male skewed sex ratio with over 80% of the initial crabs caught being male (DiBacco & Therriault 2015). This corresponded with high abundance of zoea in plankton tows and high density of gravid females (DiBacco & Therriault 2015). It is not clear why Hout Bay had a female skewed sex ratio initially, but female predominance has previously been observed in this population (T. Robinson, pers. comm). However, this trend is not common to both South African populations, as historically a male dominated population has been reported in Table Bay (T. Robinson, pers. comm). Another possible explanation for this could be environmental pollution, related to a sewage outfall pipe that discharges waste into the middle of Hout Bay. The effluent discharged from such outfalls has been linked to high levels of oestrogen (Kolpin et al. 2002), which is in turn is associated with reproductive disruption in aquatic vertebrates, leading to demasculination in fish (Vajda et al. 2011) and intersex occurrence in marine copepods (Moore & Stevenson 1991). Several endocrine disrupting effects have been observed in *C. maenas*, including morphometric, hormonal and behavioural changes as a result of sewage pollution (Lye et al. 2005). However, none of these studies have demonstrated a link between such pollution and altered sex ratios. As such, the driver behind the sex distribution of Hout Bay crabs remains unexplained.

Prior to this study, there were no quantitative data regarding *Carcinus* reproduction in Hout Bay. Based on the findings in Table Bay (see Chapter 2), reproductive individuals are found throughout most of the year, specifically from June to January. However, those results were based on a considerably smaller dataset than the current study. Overall, low numbers of gravid females were captured (a mean of 3% of females were carrying eggs) during management, an expected result as ovigerous crabs are known to show little interest in feeding (Behrens Yamada 2001, DiBacco & Therriault 2015). This is actually the highest percentage of ovigerous females captured to date in baited traps, with other

studies recording 0.25% (DiBacco & Therriault 2015) to 1% (Beal 2014). In this study, the proportions of gravid females were lowest in the months of March, April and May, the same pattern as observed in Table Bay (see Chapter 2). Ideally, management should be timed to remove the adult population prior to breeding (Genovesi 2007, Panetta 2007, Edwards & Leung 2009, Panetta & Lawes 2007, Wilson et al. 2017), however, this was not possible in Hout Bay due to the large population size and the lack of a confined pre-reproductive life history stage.

This work highlights the necessity of flexibility in invasive species management, not only in terms of administrative support, but also from a scientific perspective. The original plan for the management of *Carcinus* in South Africa was to introduce divers for collections once the catches had dropped below 10% of the maximum CPUE or daily catch recorded. In this case, the maximum CPUE was achieved on day 14, when 7.74 crabs were caught per hour of trapping. On day 33, 0.75 crabs were caught per hour. However, it took several days to assemble a dive team and once they were ready, the CPUE had increased again to 40-50% of the maximum. This increase was most likely caused by fluctuations in catches. At this point, it was decided that the 10% maximum CPUE should be sustained for 20 trapping days prior to diver introduction. The first time that this was achieved was on the 133rd day of trapping (following the management hiatus from December 2014 to January 2015). However, at this point, the work contracts of the field team had just been renewed for a further six months, therefore it was required that the trapping programme be continued until June 2015. Additionally, the costs of using a dive team over the long term were fully appreciated and a decision was made to concentrate solely on trapping.

Invasive species management programmes must continuously re-assess priorities to determine whether management actions should continue or whether resources could be better spent on improving detection rates or restoration of the ecosystem following eradication. Such decisions could include deciding when to adopt an alternative management strategy or considerations prior to the termination of management. In this case study, management ceased after 12 months. The decision to stop at this point was made on several grounds: 1) there were insufficient funds available to continue; 2) the population was much larger than previously estimated and thus management would be necessary for longer than originally anticipated; 3) the actual ecological impact of this species in the harbour environment was unknown (but have since been addressed in Chapter 4); and 4) propagule pressure was unquantified (but see Chapter 3), although a potential for immigration of crabs into Hout Bay harbour was recognised. These four factors led to the decision to abandon the extirpation effort as the uncertainty meant that the spending of additional funds could not be justified.

If the aim of management is to reduce the population over a short period of time, high effort levels would be preferable from the onset. This would likely increase the initial rate of decline of the target population, bringing the benefits of reduced impacts sooner and in

the case of *Carcinus* trapping, rapidly removing large individuals and thus those that contribute most to population reproduction. In this study, the model predicted that increasing management effort to 5,000 hours per month over a period of 18 months would result in a 21% probability of extirpation. However, the probability of success was considerably greater if the effort was further increased to 8,000 hours (with an estimated 86% probability of extirpation). Both of these scenarios were modelled at a constant level of effort from the start of the management phase, rather than involving a change in effort once management was underway. The current model is limited to a certain number of scenarios (in order to maintain mathematical convergence). Nonetheless, a management scenario with multiple effort levels would be more realistic and should be considered in future.

Insights gained from this case study

Administrative insights

One of the key attributes of a successful invasive species management programme is sufficient financial support (Myers et al. 2000). Although this is a desirable prerequisite, it is challenging to secure funds, especially when these are linked to predefined financial periods (e.g. tax years) and political objectives. Funding was made available for this work by the South African Department of Environmental Affairs, however, there is no budget available for future marine incursions. Public work programmes are typically encouraged by the South African government (McConnachie et al. 2012), as they can be used to create jobs and thus alleviate poverty while assisting with other local issues such as wildfire risk reduction and the cleaning of public spaces. A prominent example is the Working for Water Programme, which was initiated in 1995 using manual labour to clear terrestrial plant invasions (Van Wilgen et al. 1998). However, the cost-effectiveness of such programmes in terms of value to biodiversity is dubious, with the proposed ecological benefits yet to be realised (McConnachie et al. 2012). In order to accurately monitor management performance during the current study, it was necessary to record the sex and size of crabs, as well as the reproductive status of females. The data collected enabled efficient monitoring of the management effort. As a result, all the research assistants employed on the management programme required a basic biological understanding and thus, remuneration was dependent on qualification. If all research assistants (and the team manager) had a minimum qualification of a three-year graduate science degree, the cost of salaries could have been reduced to approximately ZAR 560,000 which would represent a 23.5% saving to the personnel component of the budget for managing and monitoring. However, if the effort was increased to reflect the most effective management scenario (Scenario 5 – continuous management at an increased effort level of 8,000 hours per month), even at this lower personnel rate, the budget for salaries alone would be over ZAR 2 million. This should be also considered in the context of a national

eradication programme. Hout Bay is a small harbour, but if a larger harbour were targeted (for example, Table Bay), then costs would be considerably higher. Based on the population estimates from 2013, which although inaccurate for Hout Bay, represents the most recent estimates for Table Bay, this cost would be over ZAR 50 million. If the Table Bay population were underestimated to a similar degree as Hout Bay (as extrapolated from the model), the total cost would be approximately ZAR 150 million. If both harbours were to be targeted in a nationwide eradication, the total cost (including personnel, equipment, bait etc.) at the extrapolated population size would be approximately ZAR 180 million. The lower daily personnel costs entailed during monitoring phase were the combined result of fewer staff and the assistance of several volunteers. Volunteers or citizen scientists are often utilised for conservation work in developed countries (Scyphers et al. 2015) and such programmes have been undertaken to monitor terrestrial systems in South Africa (Barnard et al. 2017). Citizen scientists were used successfully for monitoring and detecting *C. maenas* in the USA (Delaney et al. 2008). If public involvement is necessary, there are ways to ensure quality assurance and control (Robertson et al. 2010). For example, volunteers could be encouraged to collect specimens of invasive species, as well as photographic evidence with location data while experts would confirm identities (Robertson et al. 2010). On the other hand, labour costs are lower in developing countries so it is more cost-effective to run large scale long term labour-intensive programmes (Nunez & Pauchard 2010). Based on the experience gained in this project it is suggested that staff be employed where costs and circumstances allow and then volunteers be used to assist with any gaps in management. It should be acknowledged that the extrapolation of estimates from Hout Bay to Table Bay is liable to context dependency, for example, the bays might differ in landscape, habitat complexity, potential predators and site access.

This study has highlighted some of the practical difficulties of employing staff to assist with invasive species management work. Management programme teams require a leader who is responsible for co-ordination (e.g. identifying suitable candidates for the position, finalising employment contracts and applying for research permits). In this study, nobody was officially assigned this role, so it became an additional task for the lead scientist. Ensuring that the leader has the mandate and authority to make decisions is crucial. Ideally, employment contracts should be finalised at the beginning of management and linked to the proposed duration where possible. If an invasion requires a rapid response, confirmation of contracts could be challenging to organise at short notice, but every effort should be made to do so. If this is not possible, the length of contracts should be fixed so as to enable effective handover periods between staff groups. In South Africa, anybody employed for more than three months is automatically considered permanent staff (Basic Conditions of Employment Amendment Act 2002). If the duration of management programmes is increased, legally, temporary staff must be made permanent. This then has financial implications for the management authority as permanent employees are entitled to benefits. If it is necessary to maintain short contracts for these legal reasons, there

must be an adequate hand-over period to ensure that skills are not 'lost' when new staff are employed.

In South Africa, the current research permit system is very rigid: permits are issued for a maximum of twelve months and expire at the end of the calendar year. If permits could reflect the proposed project duration, this would reduce the chance of any unnecessary delays as well as reducing the administrative workload.

In theory, a break from management action (such as the six-week hiatus from December 2014 to January 2015) could compromise the integrity of an invasive species management programme. In this case, the depletion model predicted that this did not affect the probability of extirpation. This might have been influenced by two factors. Firstly, the effort level employed in the current study was far from sufficient to affect the probability of extirpation. Secondly, as the crab was able to breed throughout the year, a short break had minimal consequences. The outcome could differ for a species with a shorter generation time, as demonstrated by the attempted eradication of the invasive ascidian, *Didemnum vexillum,* in Wales (Sambrook et al. 2014). In that case, delays resulting from a lack of funding adversely affected the management outcome of the ascidian (Sambrook et al. 2014). The depletion model used in this study could be further developed to investigate the consequences of hiatuses of varying durations. However, basic project management (with the scheduling of staggered leave) would help to prevent the population from recovering during holiday periods.

Scientific insights

Most of the successful marine eradication programmes to date have targeted newly detected species (Bax et al. 2002, Wotton et al. 2004, Miller et al. 2004, Hopkins et al. 2011), with one exception where a locally established pest was discovered at an aquaculture facility and the intertidal surrounding area (Culver & Kuris 2000). If the target species is well established, there is usually no time pressure to initiate management immediately. Therefore, there is sufficient time to scientifically establish the most effective control methods, including assessment of those that have been used elsewhere as well as novel methods. This will help to ensure that methods employed are appropriate in the local context. This provides a strong basis for scientifically informed management decisions. In this case, *Carcinus* was well established and thus there was enough time to test the methods in the South African context. In contrast, if a species is recently detected, time is usually limited and thus management relies on methods tried and tested elsewhere. These might not be as effective in a different context, so there is a risk that management will be unsuccessful. Adequate monitoring will be crucial in such situations if control efforts are to be adaptive. The methods that are used in management should ideally target all reproductive life stages (Myers et al. 2000, Panetta & Timmins 2004).

Eradication might still be possible without this targeted methodology, but management goals are likely to take longer to achieve. This is more important for well-established species, such as *Carcinus* in South Africa.

Delimiting the extent of an invasion is a prerequisite for determining the effort required to remove all individuals from the population (Panetta & Lawes 2005). Aquatic species (especially those which are subtidal) can be particularly difficult to delimit due to the limited accessibility and the reliance on divers (Simberloff 2014). In South Africa, *Carcinus* appears to be restricted to two harbours and have not invaded natural ecosystems (Chapter 2). If there are limited data available regarding the delimitation of a newly detected species, management action should not be delayed. Where available, depletion models should be used to verify the population size using the data collected during management and updated as regularly as possible. This will help keep population estimates up to date and can allow for adaptive management, whereby previous experiences are used to learn and continuously adapt management plans in order to achieve a successful outcome (Lessard 1998, Roy et al. 2009).

Conclusion

In this study, while a proximate goal was to extirpate the Hout Bay population, the overall aim was to use the pilot management programme to gain an understanding of what would be required for a South Africa-wide eradication programme. Based on the results presented here, and without improved management techniques, it is recommended that the eradication of *Carcinus* from South Africa not be attempted. Considering the current distribution of this species (see Chapter 2), the complete removal of every individual nationwide will be an extremely expensive endeavour. Based on the costs associated with the mechanical removal (via baited traps) of the crab from Hout Bay and taking into account the previous underestimation of population sizes, it is estimated that to remove the species from both harbours would cost in the range of ZAR 180 million. This cost would need to be weighed against the impact of the crab as well as the ability to control potential pathways which might reintroduce the species into South Africa. In this study, at the time of the active management, the recruitment rates and the ecological impacts of this species in South Africa were unknown, and there was insufficient time to determine these factors prior to management. Given there are no indications that impacts would be severe at present (Chapter 4), that crabs might be limited to harbours (Chapter 2), that crabs will likely be re-introduced even if control was effective (Chapter 5), and the estimated costs necessary for eradication, *Carcinus* should not currently be considered a priority for management. Instead, the priority should be to prevent further spread of the species, particularly into Saldanha Bay and Langebaan Lagoon. These areas should also be prioritised for frequent monitoring of incipient *Carcinus* populations.

In conclusion, despite several challenges, this study documents the first South African attempt at managing marine invasive species. Although extirpation was not achieved, valuable insights have been gained that can be considered during future management programmes. Methodological examples from elsewhere were adapted to the local context by testing in the field. Using a model similar to those utilised in commercial fisheries, the probability of extirpation was predicted to be negligible. In retrospect, although the decision to stop management was made on administrative grounds, the estimates of this model highlight the issue with continuing management unchecked without rigorously considering the data collected. In future, such models should be incorporated into management as early as possible to allow for adaptive management and an improved probability of success.

CHAPTER 4. PREDATOR OR PREY? THE INVASIVE CRAB *CARCINUS* IN SOUTH AFRICA

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Abstract

Carcinus maenas is known for its ecological impacts in many parts of its invasive range. However, impacts of invasive species can be context dependent on both spatial and temporal scales. The potential impacts of this species were assessed in Cape Town, South Africa in 1990, shortly after its detection. The distribution of the species has changed considerably since then and a re-assessment of this species impact has become necessary. This study took advantage of a management programme targeting *Carcinus* in Hout Bay from 2014 to 2015 to assess the impacts of the crab by (1) comparing community structure through time at this harbour and (2) comparing Hout Bay (where crab numbers declined sharply) to Table Bay (where crab numbers remained stable). Sediment core samples of the benthos and vertical wall scrapes of fouling communities were collected in both harbours over a period of three years from 2014 to 2016. Tethering experiments were also conducted in Hout Bay and in a local aquarium to identify potential predators that could benefit from an additional food source presented by this crab. Benthic communities were found to be similar between harbours in 2014 (prior to the reduction of the Hout Bay crab population) but unexpectedly Hout Bay communities remained similar thereafter while Table Bay communities changed through time. In contrast, fouling communities differed between harbours at the onset of the experiment, and changed at both locations through time. As Table Bay also differed over the years, the changes observed in the Hout Bay fouling community cannot be attributed to the reduced crab density. During field tethering experiments, no crabs were attacked despite the presence of several potential predators, such as seals, sharks and seabirds. However, several native fish species (which are absent from Hout Bay harbour) preyed upon *Carcinus* under aquarium conditions, where all tethered crabs were killed shortly after deployment. This finding may explain why the distribution of the species in South Africa is largely limited to Hout Bay and Table Bay. This study highlights the role of context dependency in invasions, whereby an invasive species might have measurable ecological impacts in one region and not another.

Introduction

Introduced species often lead to shifts in the ecological balance of recipient communities (Simberloff et al. 2011), manifesting as impacts at the level of genes, individuals, populations, communities, and ecosystems (Parker et al. 1999). Such impacts can arise in marine systems through various mechanisms including competition, predation, hybridization, disease, parasitism, poisoning, bio-fouling, herbivory and interactions with other invasive species (Blackburn et al. 2014). Some species demonstrate impacts in one region but not in others, however, very few species have had impacts quantified across multiple regions (Robinson et al. 2017). In several cases, impacts have been found to be context-dependent (Paterson et al. 2015, Robinson et al. 2017), highlighting that only through the assessment of impacts of global invaders across their invasive ranges will a better understanding of the drivers of context dependency of impacts be gained. It is important to address this gap in knowledge as managers of invasive species often utilise the information gained in other regions to prioritise species for management action. Without ground truthing of region specific impacts, limited resources may be wasted on misguided management actions (Robinson et al. 2017).

The European shore crab, *Carcinus maenas*, is one of the most notorious marine invaders globally (Carlton & Cohen 2003). Compared to most marine invasive species, the impacts of *C. maenas* have been well studied, however many of these studies have considered potential ecological impacts (where the crab demonstrates potential to cause impacts) rather than documenting actual impacts in the field (for examples see Ropes 1968, Le Roux et al. 1990, Grosholz & Ruiz 1995, Grosholz et al. 2000, McDonald et al. 2001, Walton et al. 2002, De Rivera et al. 2011, Pickering & Quijon 2011, Matheson et al. 2016, Pickering et al. 2017). The potential impacts of *C. maenas* on lower trophic levels have been widely documented due to the predatory nature of this crab. The different life history stages of *C. maenas* demonstrate prey size preference with adults selecting (and successfully consuming) larger prey items (Le Roux et al. 1990). Prey selection trials conducted in the laboratory and field in North America compared predation levels and size selection between the native crab species, *Cancer irroratus,* with *C. maenas* and found the two crabs have similar dietary preferences (Mattheson & McKenzie 2014). In choice experiments using bivalves, *C. maenas* was found to prefer softshell clams (*Mya arenaria*) and blue mussels (*Mytilus edulis*) to oysters and scallops (Pickering & Quijon 2011, Mattheson & McKenzie 2014). Large *C. maenas* individuals are known to prey upon juvenile stepped venerid clam (*Katelysia scalarina*) at a higher intensity compared to native predators (Walton et al. 2002). Gut content analysis of an invasive population of *C. maenas* in North America found 88% of stomachs contained animal material (Ropes 1968). This was mostly comprised of arthropods, annelids and molluscs, with the latter being the most common (Ropes 1968). Only one third of the stomachs analysed contained plant material, suggesting that *C. maenas* is omnivorous, with a preference for animal food items (Ropes 1968).

Field studies showing actual impacts of a species are more informative than estimates of potential impacts (Kumschick et al. 2015). Those which utilise baseline data collected prior to an invasion are especially useful for comparisons with the invaded state (Kumschick et al. 2015). Unfortunately, these studies are scarce for marine invasive species. There are, however, two examples of such studies for *C. maenas* in North America (Grosholz et al. 2000, Mattheson et al. 2016). Five years after the arrival of *C. maenas*, a five-fold decline in density of two native clam species (*Nutricola tantilla* and *N. confusa*) was observed (Grosholz et al. 2000). In addition, the abundance of the native crab, *Hemigrapsus oregonensis*, declined approximately ten-fold post-invasion (Grosholz et al. 2000). Another study relied on the use of pre- and post-invasion surveys of eelgrass (*Zostera marina*) beds. This work demonstrated that five years after the initial detection of *C. maenas*, eelgrass abundance declined by 50%, with some sites experiencing total losses of eelgrass (Mattheson et al. 2016). *Carcinus maenas* is known to exert this impact on eelgrass beds through juvenile grazing and the uprooting of shoots by adults (Malyshev & Quijon 2011). In addition, declines in fish biomass and abundance have been recorded (Matheson et al. 2016), highlighting the importance of eelgrass as a habitat for juvenile fish (Laurel et al. 2003, Robichaud & Rose 2006), and the cascading impacts of this crab.

Seabirds and other crab species are known to prey upon *C. maenas* in North America (Dumas & Witman 1993, Hunt 2001, Jensen et al. 2007, Schafer 2008). In its non-native Australian range, Garside et al. (2015) found the abundance of *C. maenas* to be negatively correlated with the abundance of six native species, including a crab (*Portunus pelagicus*), an octopus (*Octopus* sp.), and fish (*Monocanthidae, Acanthopagrus australis, Tetraodontidae,* and *Trygonorrhina fasciata*). Feeding trials confirmed that the crab and octopus were capable of eating *C. maenas*, suggesting that these species may prey upon *C. maenas* in the wild (Garside et al. 2015). Despite the negative correlation, the fish species were most likely limited by the crab size, potentially capable of preying upon small individuals only (Garside et al. 2015).

Given the actual ecological impacts of *C. maenas* invasions elsewhere, it is possible that similar impacts might occur in South Africa. A previous South African study has described potential impacts of *C. maenas* on gastropods, isopods and polychaetes from rocky intertidal shores (Le Roux et al. 1990). However, to date, no study has investigated actual impacts in the subtidal zone, the environment in which this species predominantly occurs in South African waters (See Chapter 2). As such, an opportunity exists to assess the impacts of this predator within South African subtidal habitats. In addition, the potential role of *Carcinus* as prey for native predators remains unknown in South Africa. This chapter thus aims to establish the impact of this crab on the subtidal communities on which it is thought to prey while also considering its role as a food source to native predators. Based on the lower trophic impacts reported from other regions (Grosholz & Ruiz 1995, Mattheson et al. 2016) and evidence suggesting this crab is preyed upon by higher trophic level native predators elsewhere (Dumas & Witman 1993, Schafer 2008,

Garside et al. 2015), two *a priori* hypotheses were tested. Firstly, it was hypothesised that *C. meanas* would alter the structure and diversity of subtidal benthic and fouling communities and secondly, it was hypothesised that native predators such as predatory fish and diving sea birds would consume this invader.

Methods

Impacts of Carcinus *on subtidal community structure and diversity*

A pilot management programme was initiated in Hout Bay harbour in 2014 to control the *Carcinus* population (see Chapter 3 for further details). This provided a unique opportunity to consider the structure of potential prey communities present prior to and following the management of *Carcinus*, thus enabling the isolation of the impact of this crab on these communities. A Before-After Control-Impact framework (Underwood 1992, Underwood 1994) was used to examine the impacts of *Carcinus* on the subtidal community structure in Hout Bay harbour. The 'before' samples were collected in the harbour in 2014, prior to initiation of the management programme while the 'after' samples were collected during and post-management (in 2015 and 2016 respectively). A 'control' site (at the Royal Cape Yacht Club in Table Bay harbour) was compared to the 'impact' site (Hout Bay harbour) over the same time-frame.

Table Bay and Hout Bay harbours represent the only locations in South Africa that are currently invaded by *Carcinus*. As Table Bay is much larger than Hout Bay harbour, a single yacht basin (Royal Cape Yacht Club), that closely matches Hout Bay harbour in habitat type and traffic characteristics, was chosen as a reference site. These sites are both frequented by recreational sailing vessels offering areas of low water movement with sandy bottoms and vertical cement wharfs (T. Robinson, pers. obs. following pilot surveys). At both sites, samples were collected in April of each year to avoid seasonal variability. On each sampling occasion, scientific SCUBA divers collected five benthic core samples, each with a diameter of 15 cm, approximately 15 m apart. Cores samples were extended to a depth of 30 cm. In addition, five vertical fouling scrapes were collected using a 25 cm x 25 cm quadrat. Vertical scrapes were collected 1 m above the sediment at 15 m intervals. The number of samples collected were limited by the logistical constraints of sampling two habitats in two harbours across three years. All samples were sieved using a 1 x 1 mm sieve before being stored in plastic containers and preserved with 95% formalin. Organisms were identified to the lowest taxonomic level possible and the biomass of each species per sample was measured to the nearest 0.01 g. Biomass was used as a measure of abundance as it was not possible to count individuals of some species post-collection (e.g. barnacles that were unavoidably damaged during the removal of scrape samples).

Community composition was compared between sites (Fixed factor: Table Bay and Hout

Bay) and among years (Random factor: 2014, 2015 and 2016) using PERMANOVA in PRIMER version 6 (Clarke & Gorley 2006, Anderson et al. 2008). In order to weight the contribution of species supporting high biomasses, data were fourth-root transformed (Field et al. 1982, Clarke et al. 2014). Samples were classified by a hierarchical clustering method using the Bray-Curtis similarity index and group average linkage. In order to determine which species were responsible for dissimilarities identified by the PERMANOVA, an analysis of similarities based on percentages contributed was performed using SIMPER. While five samples were collected per site each year, in 2014 only four core samples and two scrape samples from Hout Bay could be analysed as the other samples decomposed before they could be processed. In addition, three samples were excluded from the community composition analyses as each contained only a single species not recorded in any other samples.

The Shannon-Weiner diversity index (a measure of incorporating species richness and evenness) was used as a measure of diversity and calculated using the following equation:

$$H = -\Sigma ipi(\ln pi)$$

Where pi is the proportion of the total biomass arising from a particular species (species i) and H is the sum of biomass of all species collected in a sample. This measure was examined with respect to site (two levels: Hout Bay and Table Bay) and year (three levels: 2014, 2015 and 2016) in a two-factor generalised linear model with a quasipoisson error distribution. All univariate statistics were conducted in the R statistical environment using the basic statistical package (version 3.3.0, R core team, 2016).

Predation on Carcinus

Experiments involving enclosures or tethering allow for an estimation of predation potential or predator-prey encounters. This can be calculated using the frequency of attacks, whereby the subject is injured or killed (Aronson 1989). However, the results of these experiments must be interpreted carefully as effects can be overestimated due to the imposed constraints of movement on the tethered organism (Peterson & Black 1994).

Tethering experiments were undertaken to determine potential predators of *Carcinus.* Crabs were collected in Hout Bay using baited traps (See Chapter 2 and Figure 2.4 for a detailed description) then sexed and sized based on carapace width and placed into four size categories (small ≤40 mm; medium 41-60 mm; large 61-80 mm and extra-large ≥81 mm). Six replicates per sex per size class were used with the exception of the extra-large size class as no females ≥81 mm were collected in the traps. All crabs were in non-moulting condition with both chelipeds and all walking legs present. Crabs were tethered using 50 cm of monofilament line (tension 10 kg) attached to the carapace with cyanoacrylate adhesive glue. After attachment of the tether, individuals were kept under

laboratory conditions (10-12°C water temperature) for 24 hours to allow for acclimation and to confirm that there were no observable adverse effects (behavioural or physiological changes) resulting from the tethering process. Crabs were tethered to a metal frame (Figure 4.1) and then deployed for a period of three hours. A GoPro video camera was fixed to the frame, 1 m from the crab anchoring point, at a height of 0.3 m above the substrate.

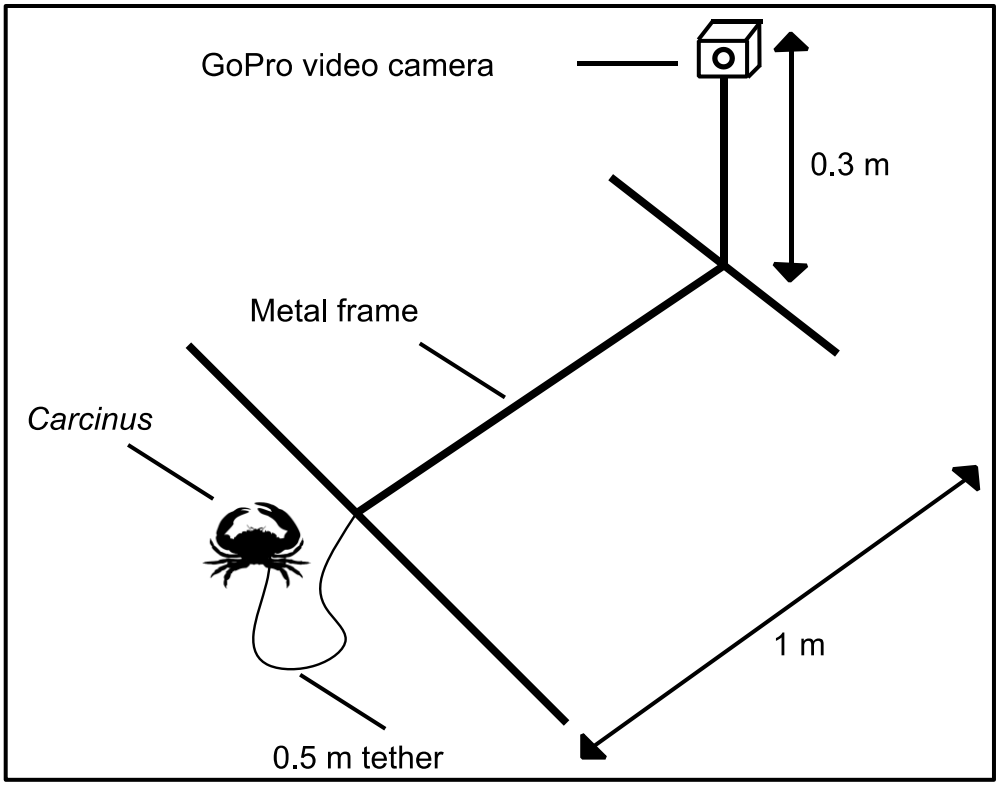

Figure 4.1. Tethering set up used in experiments to determine potential predators of *Carcinus*.

The time of experiment initiation was recorded as the point at which the metal frame was placed on the substrate. The total number of attacks was recorded with each attack defined as a single biting action on the crab body by any individual organism. The time to fatal attack was taken to be the time from the start of the experiment until the tether was broken. All species that approached within 30 cm of the tethered crab (thereby demonstrating interest), as well as those that attacked the crab were recorded. Attacks were noted as non-fatal or fatal. The behavioural response of the crab to potential predators was recorded as none (crab remained still), passive (attempted to find shelter) or aggressive (meral spread whereby the body is raised and chelipeds held out to defend the crab against predators).

Initial tethering experiments were conducted in Hout Bay harbour after which these experiments were replicated in the 'Kelp Forest' tank at the Two Oceans Aquarium in Cape Town. While an ideal experimental design would have seen these trials replicated in a natural habitat outside of the harbour, the placement of a legally regulated alien species into an area in which it does not presently occur is prohibited in South Africa (NEMBA Alien and Invasive Species Regulations 2014). As such, the aquarium offered a controlled model system containing the native faunal composition as would be expected to occur in subtidal habitats along the Cape Peninsula. The tank volume was 800,000 litres and the benthic surface was comprised of mixed hard and soft substrate. Due to the unavailability of crabs larger than or equal to 81 mm, the aquarium experiments excluded both males and females in this size class.

The tethering data were extracted from the video footage. The number of interested predatory species were examined with respect to sex (two levels: male and female) and size category (four levels: small, medium, large and extra-large) of the crabs in a two-factor generalized linear model, using a quasipoisson error distribution. Post hoc analyses were conducted using pairwise t-tests with Bonferroni corrections. The frequency of behavioural response (three levels: none, passive and aggressive) of the tethered crab was analysed per size category (four levels: small, medium, large and extra-large) using a Pearson's Chi Squared test. Similarly, the time from the setting of the trap to the first and fatal attack, as well as the total number of attacks were examined with respect to sex (two levels: male and female) and size category (four levels: small, medium, large and extra-large). In the aquarium trials, only three size levels were considered in each model (i.e. small, medium and large).

Results

Impacts of Carcinus *on subtidal community structure and diversity*

In total, 50 species from 40 genera were recorded in benthic and fouling communities. Benthic communities differed significantly among years and harbours with a significant interaction between site and year (Table 4.1, Figure 4.2). Pairwise comparisons showed that Table Bay and Hout Bay communities were not significantly different in 2014 but this changed in 2015 and 2016. However, when considering changes in community structure through time at each harbour, it was found that Hout Bay benthic communities remained the same across years while those in Table Bay showed temporal variation.

Table 4.1. PERMANOVA results (main test and pairwise tests) from the benthic communities collected in Table Bay and Hout Bay harbours in 2014, 2015 and 2016. * indicates a significant result. For more detailed test results, see Appendix 4.1 and 4.2.

Factor	df	Pseudo-F	*p*
Site	1	6.3399	0.001*
Year	2	3.0665	0.001*
Site x Year	2	2.8073	0.001*
Site x Year: 2014	1	1.3678	0.090
Site x Year: 2015	1	2.1506	0.009*
Site x Year: 2016	1	2.6887	0.011*
Site x Year: Hout Bay			
2014, 2015	1	0.5608	0.899
2014, 2016	1	0.9167	0.589
2015, 2016	1	1.0550	0.364
Site x Year: Table Bay			
2014, 2015	1	2.5660	0.008*
2014, 2016	1	2.2148	0.009*
2015, 2016	1	4.1049	0.008*

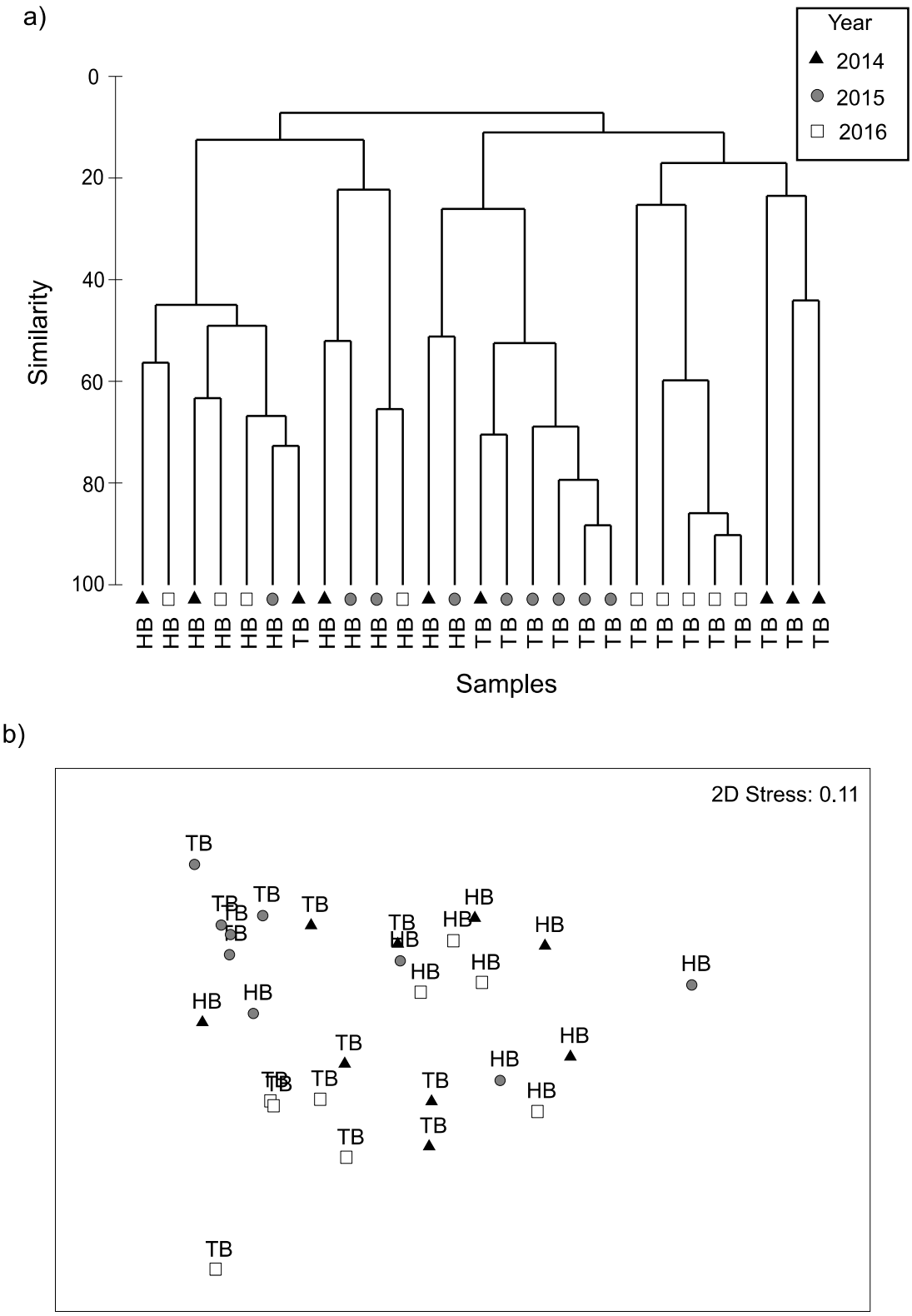

Figure 4.2. a) Dendrogram and b) Two dimensional MDS ordination plot showing hierarchical cluster analysis using the Bray-Curtis similarity index of benthic communities in Table Bay (TB) and Hout Bay (HB) harbours in 2014, 2015 and 2016.

The SIMPER analysis of the benthic communities revealed that four species contributed approximately 51% to the differences between the sites. The most important being the native sea cucumber, *Thyone aurea,* which was only found in Table Bay (Figure 4.3). The alien mussel, *Mytilus galloprovincialis,* was found in both harbours but had the highest abundance in Hout Bay. In contrast, the native polycheate, *Lumbrineris tetraura,* was more abundant in Table Bay than Hout Bay harbour while the alien ascidian, *Ciona robusta* was only present in Table Bay.

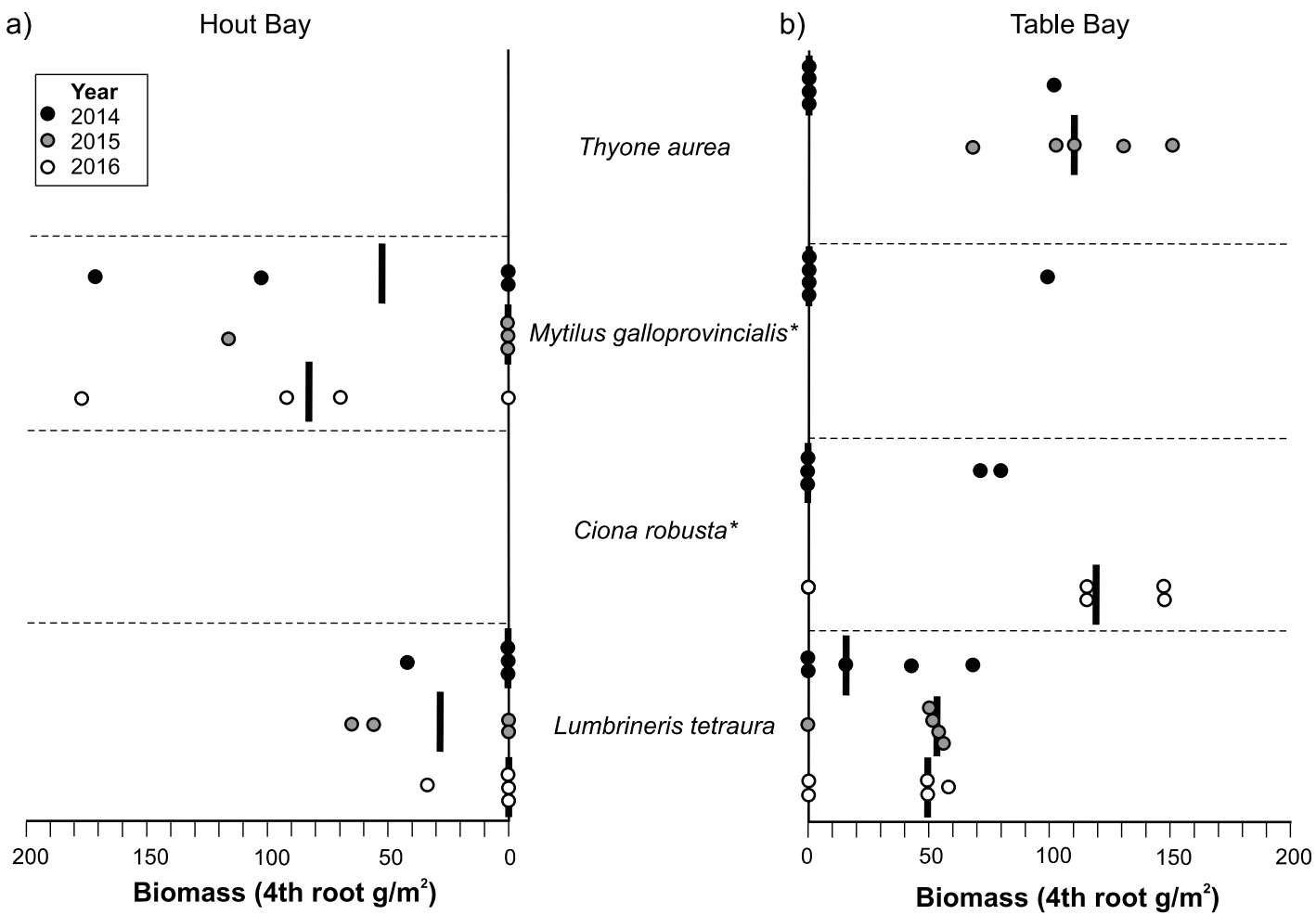

Figure 4.3. Fourth root transformed biomass (g per m^2) of the top four species responsible for the dissimilarities between the a) Hout Bay and b) Table Bay benthic communities for each year sampled. The median is shown with a solid black bar, while an asterix identifies species that are recognised as invasive in South Africa (Robinson et al. 2016).

Benthic community diversity differed significantly between the two harbours but not among years (Table 4.2, Figure 4.4).

Table 4.2. GLM results of benthic community diversity with respect to site (two levels: Hout Bay and Table Bay) and year (three levels: 2014, 2015 and 2016). * indicates a significant result. For more detailed test results, see Appendix 4.3.

Factor	df	F	*p*
Site	1	8.252	0.009*
Year	2	1.705	0.205
Site x Year	2	0.999	0.328

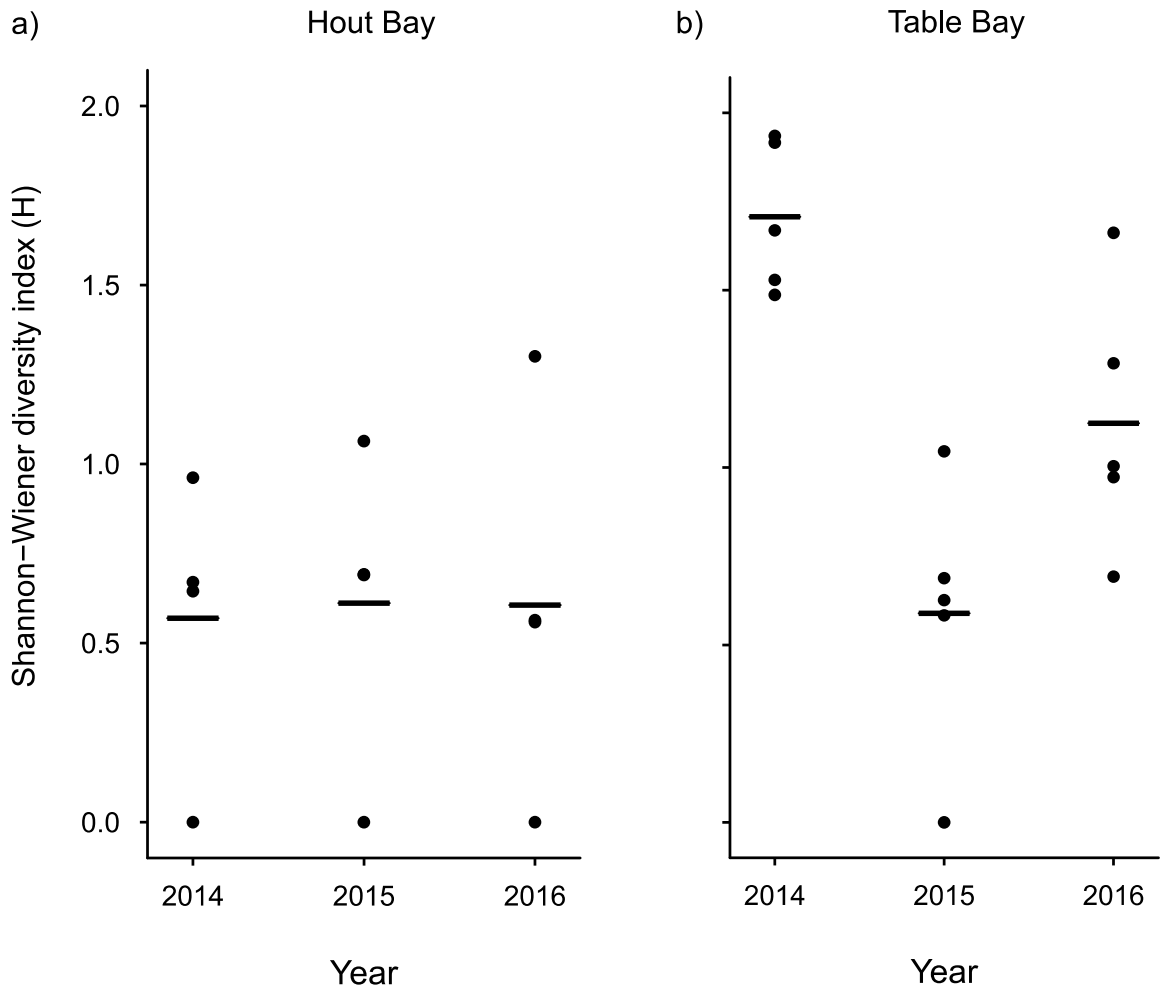

Figure 4.4. Scatterplots showing mean Shannon-Wiener diversity index (horizontal line) and raw data for the benthic communities per year for (a) Hout Bay and (b) Table Bay. The effect of year was non-significant (*p* > 0.05), while diversity differed significantly between harbours (*p* = 0.009).

Fouling community structure differed significantly between harbours and among years with a significant interaction between these factors (Table 4.3, Figure 4.5). However, unlike the benthic communities, the pairwise tests showed that the harbours were significantly different in each year that they were sampled. Fouling communities in Hout Bay harbour demonstrated a progressive change through time (as demonstrated by the lack of difference between 2014 and 2015, yet a significant difference between 2014 and 2016). The fouling community structure in Table Bay was significantly different every year.

Table 4.3 PERMANOVA results (main test and pairwise tests) from the fouling communities collected in Table Bay and Hout Bay harbours in 2014, 2015 and 2016. * indicates a significant result. For more detailed test results, see Appendix 4.4 and 4.5.

Factor	df	Pseudo-F	*p*
Site	1	16.1520	0.001*
Year	2	9.2176	0.001*
Site x Year	2	3.5342	0.001*
Site x Year: 2014	1	2.8240	0.045*
Site x Year: 2015	1	2.6672	0.004*
Site x Year: 2016	1	3.1874	0.005*
Site x Year: Hout Bay			
2014, 2015	1	1.1920	0.262
2014, 2016	1	2.5448	0.036*
2015, 2016	1	3.0454	0.008*
Site x Year: Table Bay			
2014, 2015	1	3.2375	0.007*
2014, 2016	1	3.1584	0.008*
2015, 2016	1	2.0893	0.012*

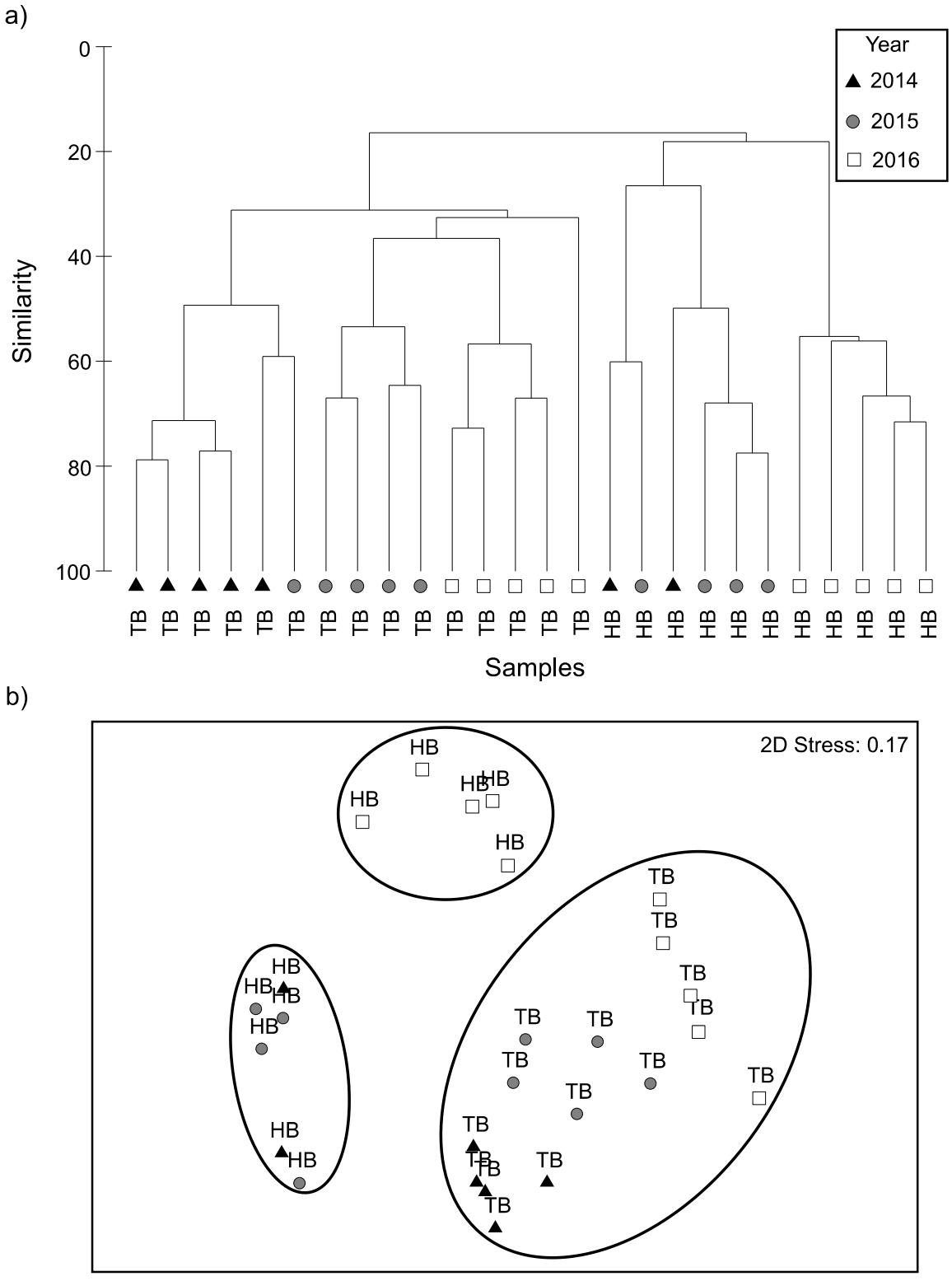

Figure 4.5. a) Dendrogram b) Two dimensional MDS ordination plot showing hierarchical cluster analysis using the Bray-Curtis similarity index of benthic communities in Table Bay (TB) and Hout Bay (HB) harbours in 2014, 2015 and 2016. The circles demonstrate similarities in the MDS plot.

The SIMPER analysis of the fouling communities revealed that four species were responsible for 34% of the differences between the sites. The most important of these species was *C. robusta,* with the highest abundance observed in Table Bay (Figure 4.6). In both harbours, there were several mussel recruits (which could not be identified to species level due to recruits measuring < 5mm) and these, along with the mussel *Mytilus galloprovincialis,* were most abundant in Hout Bay. A third species, the native mussel, *Aulacomya atra*, was most abundant in Table Bay.

In contrast to the benthic community results, the diversity of fouling communities showed no main effects of site or year but rather an interaction between these predictors (Table 4.4, Figure 4.7).

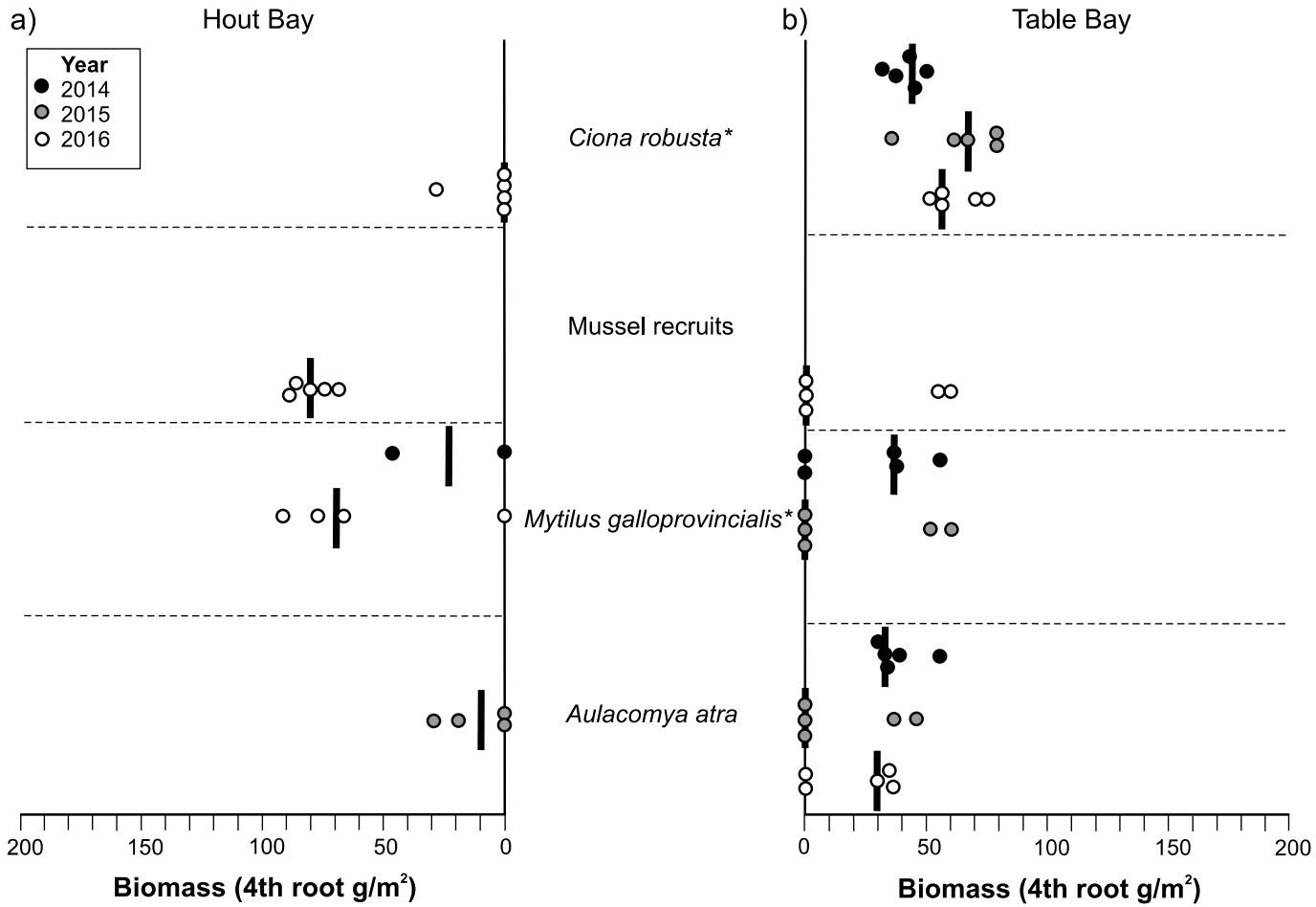

Figure 4.6. Fourth root transformed biomass (g per m^2) of the top four species responsible for the dissimilarities between the a) Hout Bay and b) Table Bay fouling communities for each year sampled. The median is shown with a solid black bar, while an asterix following the species name indicates its invasiveness in South Africa.

Table 4.4. GLM results of fouling community diversity with respect to site (two levels: Hout Bay and Table Bay) and year (three levels: 2014, 2015 and 2016). * indicates a significant result. For more detailed test results, see Appendix 4.6.

Factor	df	F	*p*
Site	1	1.2297	0.2794
Year	2	0.7425	0.3981
Site x Year	2	13.1266	0.0015*

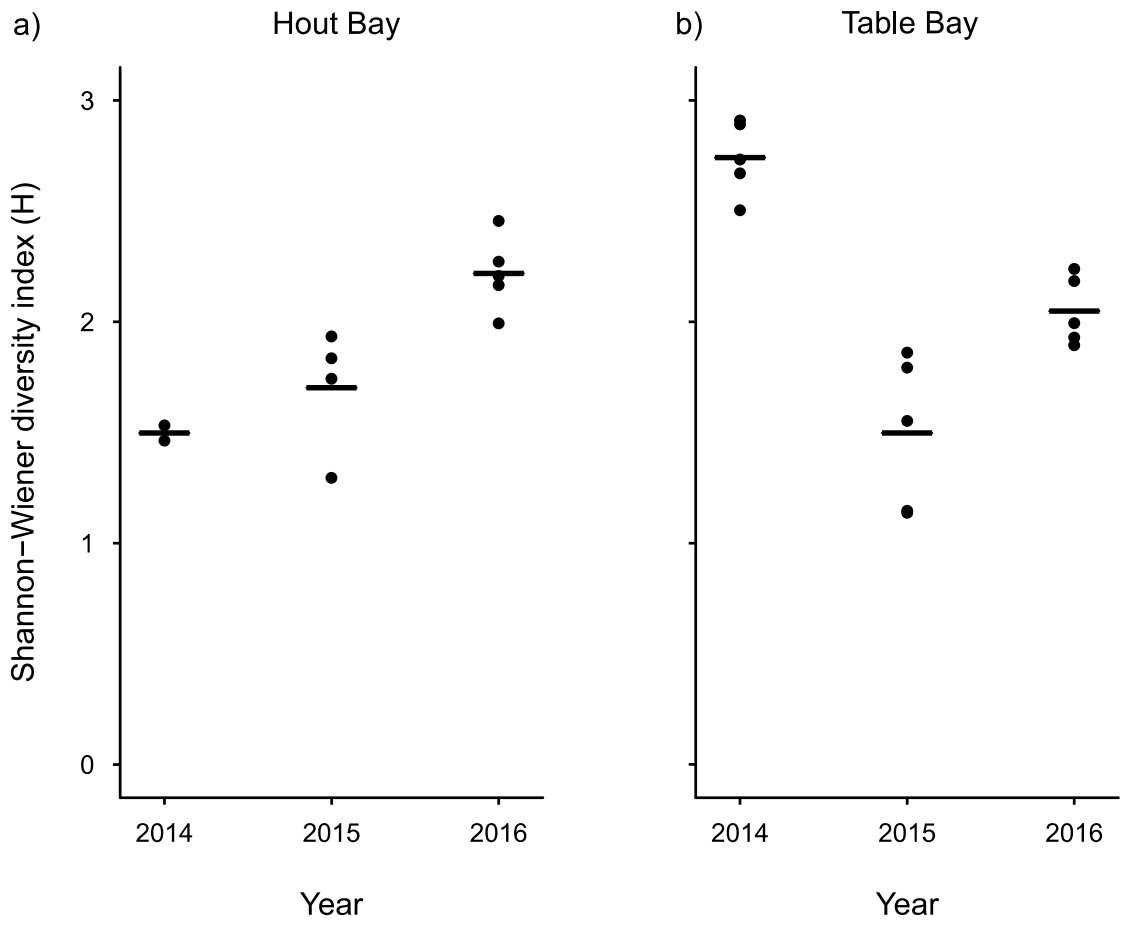

Figure 4.7. Scatterplots showing mean Shannon-Wiener diversity index (horizontal line) and raw data for the fouling communities per year for (a) Hout Bay and (b) Table Bay. All main effects were non-significant ($p > 0.05$).

Predation on Carcinus

None of the tethered crabs in Hout Bay harbour were preyed upon. Several potential predators were identified from the video footage, including the South African fur seal *Arctocephalus pusillus*, cormorant *Phalacrocorax* sp. and shyshark *Haploblepharus* sp.

However, none of these potential predators showed any interest in the tethered crabs during the 126 hours of observations.

In the aquarium experiment, all of the tethered crabs were consumed by predatory fish. The most common predator was the Red stumpnose, *Chrysoblephus gibbiceps,* which killed 21 of the 36 crabs (58% of the total number tethered). This species was the second most common in the tank, with a relative density of 0.17. Other species that attacked tethered crabs are listed in Table 4.5. The sex of tethered crabs did not affect the number of species which demonstrated interest, however, there was a significant effect of size (Table 4.6, Figure 4.8). Significantly more species were interested in medium sized crabs than small crabs (t = 2.138, p = 0.041). Interestingly, there was no significant effect of sex or size of the crabs on the time which elapsed between deployment and the first attack (Table 4.7, Figure 4.9a). The mean time from the start of the experiment to a fatal attack was 72 seconds (95% CI: 47-96 seconds) with the longest survival time being 321 seconds (i.e. 5.35 minutes). There was no significant effect of sex and size on this measure (Table 4.7, Figure 4.9b). The size of the crabs affected the number of attacks experienced (Table 4.8, Figure 4.9c) and post-hoc tests revealed that medium crabs were more likely to be attacked than crabs in the smallest size category (t = 2.138, p = 0.041).

Table 4.5. Species which attacked the tethered crabs, the relative density per m^2 and the type of attack witnessed (non-fatal or fatal).

Species	Common name	Relative density (per m^2)	Attack type (non-fatal/fatal)
Boopsoidea inornata	Fransmadam	0.20	Non-fatal
Chrysoblephus cristiceps	Dageraad	0.01	Fatal
Chrysoblephus gibbiceps	Red stumpnose	0.17	Non-fatal; Fatal
Chrysoblephus laticeps	Red roman	0.04	Non-fatal; Fatal
Cymatoceps nasutus	Black musselcracker	0.01	Fatal
Dichistius capensis	Galjoen	0.14	Non-fatal
Diplodus hottentotus	Zebra	0.12	Non-fatal
Epinephalus marginatus	Yellow belly rockcod	0.04	Non-fatal
Pachymetopon blochii	Hottentot	0.11	Non-fatal
Pachymetopon grande	Bronze bream	0.03	Non-fatal; Fatal
Sarpa salpa	Strepie	0.07	Non-fatal
Spondyliosoma emarginatum	Steentjie	0.04	Non-fatal

Table 4.6. GLM results of the number of interested species with respect to sex (two levels: Male, Female) and size class (three levels: Small, Medium, Large). Size classes were assigned based on carapace width: small (≤40 mm); medium (41-60 mm) and large (61-80 mm). * indicates a significant result. For more detailed test results, see Appendix 4.7.

Factor	df	F	*p*
Sex	1	0.1227	0.7286
Size class	2	5.2856	0.0108*
Sex x Size class	2	2.1104	0.1388

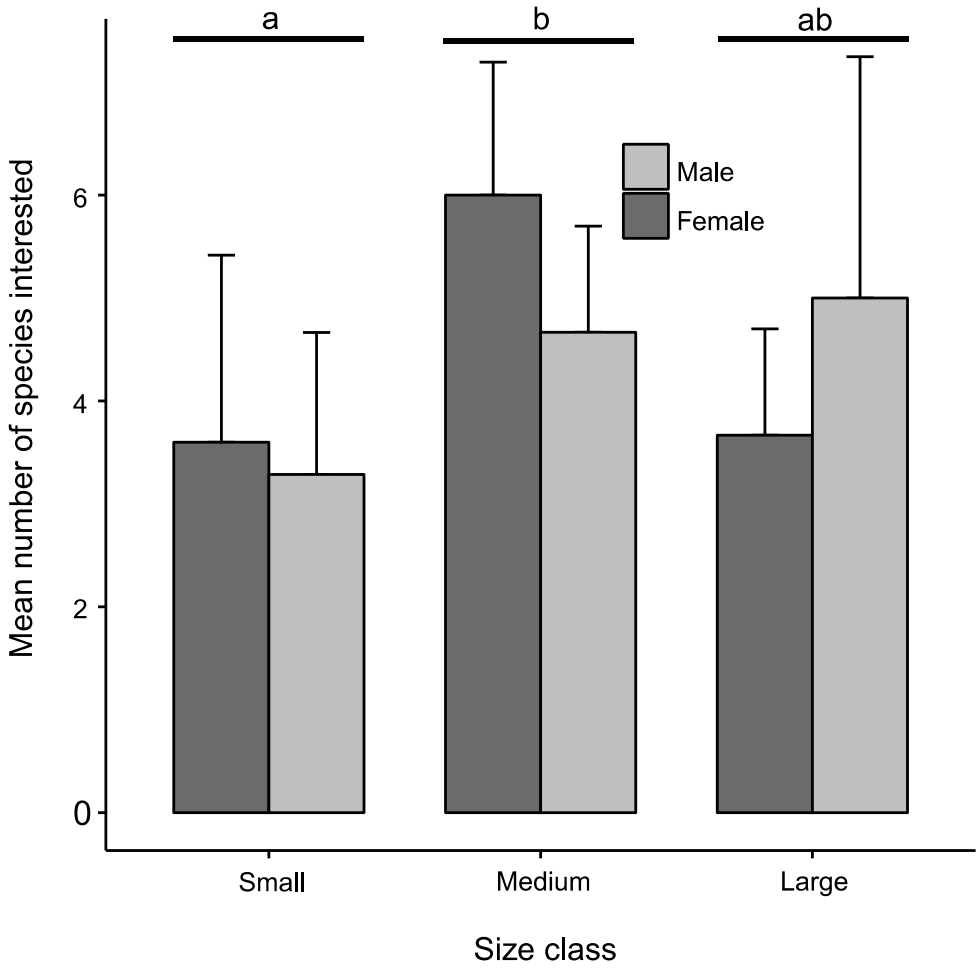

Figure 4.8. Mean (± SD) number of species interested in tethered male and female crabs of each size class (t = 2.498, *p* = 0.018). Size classes were assigned based on carapace width: small (≤40 mm); medium (41-60 mm) and large (61-80 mm). The lettering refers to the main effect of size with size classes sharing lettering not significantly different from each other (*p* > 0.05).

Table 4.7. GLM results of a) the time to the first attack with respect to sex (two levels: Male and Female) and size class (three levels: Small, Medium and Large) and b) the time to the fatal attack with respect to sex (two levels: Male and Female) and size class (three levels: Small, Medium and Large). Size classes were assigned based on carapace width: small (≤40 mm); medium (41-60 mm) and large (61-80 mm). For further details, see Appendix 4.8.

Factor	df	F	*p*
a) *Time to first attack*			
Sex	1	1.2291	0.2764
Size class	2	1.1025	0.3451
Sex x Size class	2	2.0970	0.1405
b) *Time to fatal attack*			
Sex	1	0.0488	0.8266
Size class	2	1.1601	0.3271
Sex x Size class	2	1.7582	0.1896

Table 4.8. GLM results of the number of attacks with respect to sex (two levels: Male and Female) and size class (three levels: Small, Medium and Large). Size classes were assigned based on carapace width: small (≤40 mm); medium (41-60 mm) and large (61-80 mm). * indicates a significant result. For more detailed test results, see Appendix 4.9.

Factor	df	F	*p*
Sex	1	2.8634	0.1010
Size class	2	8.6371	0.0011*
Sex x Size class	2	2.1231	0.1373

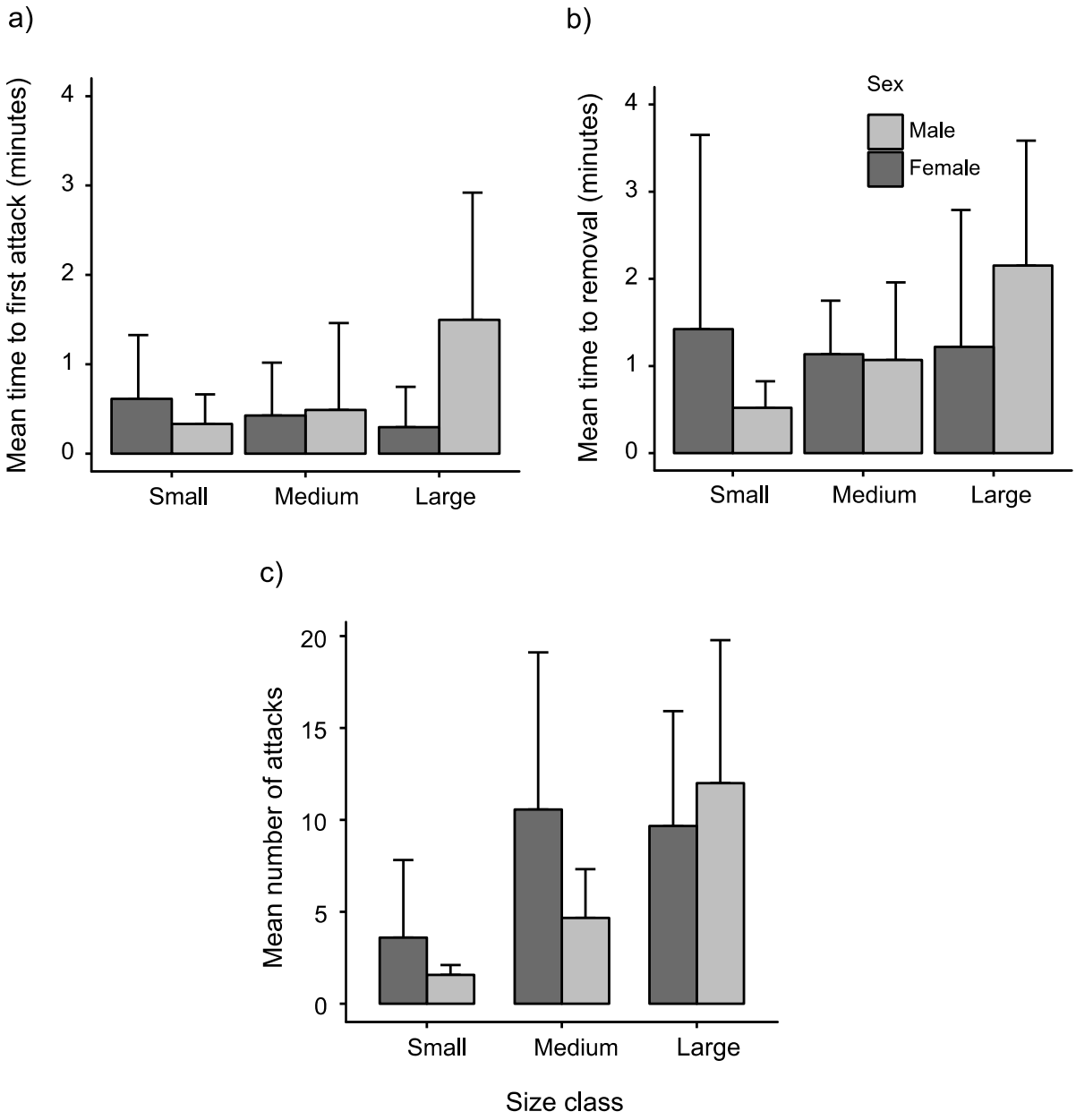

Figure 4.9. a) Mean time (± SD) to first attack of male and female crabs in each size class. All differences were non-significant ($p > 0.05$); b) Mean time (± SD) to fatal attack or removal of male and female crabs in each size class. All differences were non-significant ($p > 0.05$); c) Mean number (± SD) of attacks on male and female crabs according to the size class (F=8.637, p =0.001). Size classes were assigned based on carapace width: small (≤40 mm); medium (41-60 mm) and large (61-80 mm).

There was no significant effect of size class of the tethered crab on the frequency of behavioural response to an interested potential predator (X^2 = 18, df = 14, p = 0.21). The most frequently observed behaviour was for the tethered crab to ignore interested fish species, with an aggressive response also commonly observed (Figure 4.10).

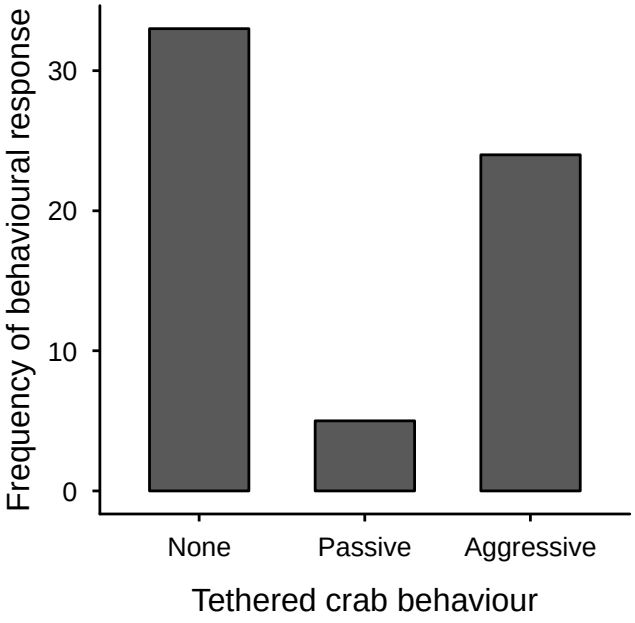

Figure 4.10. Frequency of behavioural response (categories: none, passive or aggressive) by tethered crabs to interested potential predators in the aquarium.

Discussion

The extent of ecological impacts caused by an invasive species is often used to justify management actions (Blackburn et al. 2014). However, these impacts can be context dependent (Thomsen et al. 2011, Blackburn et al. 2014, Kumschick et al. 2015, Ojaveer et al. 2015, Robinson et al. 2017). The ecological impacts associated with *Carcinus maenas* across its global invasive ranges have been the focus of many studies (Ropes 1968, Le Roux et al. 1990, Grosholz & Ruiz 1995, Grosholz et al. 2000, McDonald et al. 2001, Walton et al. 2002, De Rivera et al. 2011, Pickering & Quijon 2011, Matheson et al. 2016). The potential impacts of this crab in South Africa have been documented (Le Roux et al. 1990) but, to date, there has been no measure of realised impacts. This study found no evidence of ecological impacts of *Carcinus* in Hout Bay, refuting the a priori hypothesis that as in other invaded regions this crab has measurable effects on community structure. However, it should be noted that this study only assessed one measure of ecological impacts. The benthic communities in the harbour were not significantly impacted by the reduction of the *Carcinus,* and while the fouling communities in Hout Bay differed through time the mechanism behind these changes could not be ascribed to *Carcinus* as the Table Bay fouling communities also changed over the same period. Tethered crabs within the harbour were not preyed upon despite the presence of potential predators. This was not the case in the aquarium tethering experiment where the crabs were eaten by a variety of predatory fish.

Community analyses demonstrated that benthic communities were comparable between Table Bay and Hout Bay harbours in 2014. The Table Bay benthic communities were dynamic across the years while those in Hout Bay remained relatively stable, despite the reduction of the *Carcinus* population in Hout Bay between 2014 and 2015. This suggests that the crabs are not driving the structure of communities through the predation of benthic organisms or modification of the benthic habitat (e.g. by burrowing into the sediment). Interestingly, in the crab's native range, predation and sediment modification, as a result of crab activity, is known to alter nematode assemblages (Schratzberger & Warwick 1999), highlighting its role regulating benthic communities. It is notable that nematodes were absent from the samples collected in the present study (most likely due to the mesh size used) so no conclusions can be drawn with regards to this functional group.

In contrast to the benthic communities, the fouling communities showed a significant effect of site and year. As both harbours showed changes in community composition through time, it is challenging to draw inferences from these data. There may be an effect of management efforts in Hout Bay and the resulting decrease in the *Carcinus* population, however, these results are inconclusive. Additionally, considering changes in abundance of key species offers no resolution. Of the top four species contributing most to the differences between Table Bay and Hout Bay, the invasive mussel *Mytillus galloprovincialis* and the native mussel, *Aulacomya atra,* were present in 2014 and 2015 but absent from Hout Bay in 2016. *Carcinus maenas* is known to prey upon bivalves, including the congener of *M. galloprovincialis*, *M. edulis* in its native range (Elner 1978). If *Carcinus* were to prey upon *M. galloprovincialis*, removal of the crab may result in an increased abundance of the mussel. However, given that *M. galloprovincialis* appears to have decreased since the management of *Carcinus*, it is unlikely that the crab is impacting the mussel population in Hout Bay.

At present, the *Carcinus* invasion is limited to the artificial harbour environments of Hout Bay and Table Bay, however, if the species spread into natural habitats, a different result may be seen. The North American studies that demonstrated actual impacts of the crab were conducted in natural habitats and relied on true pre- and post-invasion datasets gathered over a long time period (Grosholz et al. 2000, Matheson et al. 2016). The scenario created by the crab reduction in Hout Bay harbour is unlikely to emulate the pre-invasion environmental state. In addition, this study considered impacts over a relatively short time frame following the reduction in the crab population, so it is possible that there may have been insufficient time passed to observe an actual impact on the community structure in Hout Bay. While, marine communities have been known to respond to modifications in their environment over short periods (Wahl et al. 2011), longer-term studies than the present may offer further resolution. Future research could also involve the varying of crab densities (under controlled conditions) to assess the impacts on communities in response to different crab abundances. This may help to identify a critical density threshold whereby impacts of the crab are relaxed or amplified.

In South Africa, the distribution of *Carcinus* is currently confined to the two harbours included in this study (see Chapter 2). The fact that no evidence of impact by *Carcinus* on communities in the harbour environments has been found, does not necessarily mean it would have no impact outside of these disturbed environments. Generally, the communities living in harbour environments are exposed to high levels of pollutants and organic enrichment (Fatoki et al. 2012) and thus could be expected to be more resistant to impacts from introduced species. Both Hout Bay and Table Bay have fish processing plants within the harbours. These factories regularly dump fish offal into the harbour water (C. Mabin, pers. obs.). In addition, many fishing boats return to Hout Bay every day and discard unwanted fish waste and bait overboard. This readily available food source likely offers a constant and energy efficient food source to the crabs, potentially reducing the need for the crabs to prey upon native flora and fauna. Indeed, the areas in Hout Bay harbour which are used for regular factory waste disposal correlated with the highest abundance of *Carcinus* (see Chapter 3 for further details). Given that these harbours cannot be classed as natural habitats and indeed a number of the species inhabiting these are alien, any changes detected in the communities should be considered the result of novel ecological interactions rather than impacts per se.

Although a rare occurrence, some invasive species show significant impacts early in their invasion history, which may decline over time (Morrison 2002, Strayer et al. 2006, De Rivera et al. 2011). This may be linked to boom-bust dynamics, with abundance of the invasive species directly related to impacts (Simberloff & Gibbons 2004, Crooks 2005). A long-term tethering study in Bodega Bay revealed multiple impacts (over a period of 14 years) of the invasive *C. maenas* on the native shore crab, *Hemigrapsus oregonensis,* prior to, during and following management action (De Rivera et al. 2011). Abundance of the native crab was negatively correlated with the non-native species for thirteen years (De Rivera et al. 2011). In the earlier years of the invasion, *H. oregonensis* decreased markedly in body size and shifted its distribution into the higher intertidal zone (De Rivera et al. 2011). When the *C. maenas* population experienced a decline, *H. oregonensis* numbers increased to pre-invasion levels following a lag period (De Rivera et al. 2011). However, the changes in body size and tidal distribution of the native crab persisted for a further ten years with *H. oregonensis* reaching pre-invasion levels towards the end of the study (De Rivera et al. 2011). There is no native crab in South Africa analogous to *C. maenas* (Le Roux et al. 1990) and thus the crab may have novel influences in this system.

During the field tethering experiments, none of the tethered crabs were damaged or fatally attacked, despite the presence of potential predators in Hout Bay. This could mean that the predatory animals in the harbour were not interested in the crabs as a food source, or they were adequately satiated during the experiment. The fish processing plants and fishing vessels in the harbour provide a source of food with minimal handling time and risk of injury for these potential predators as well as the crabs. However, in natural areas outside of the

harbour, predators do not have access to such a consistent food source and thus could well react in a similar way to that recorded in the aquarium experiment.

The results from the experiment in the kelp forest tank in the aquarium should be viewed in context. As an aquarium exhibit, this is technically an artificial environment with the relative densities of fish in the tank being considerably higher than the densities observed in the wild along the South African coast (De Vos et al. 2014, Roberson et al. 2015). However, the results can be indicative of which species of fish could act as potential predators of *Carcinus* in the wild. Only 5 out of 12 fish species were observed committing fatal attacks. These five species may have been the dominant fish in the aquarium, intimidating other species from attacking the tethered crabs. There was no significant effect of crab size or sex on the time which elapsed between deployment and the first attack or removal of the crab. However, there was an effect of size on the total number of attacks on the crabs, with the intermediate size class experiencing more attacks than the smallest crabs. These crabs tended to be more aggressive in behaviour and thus more minor attacks were necessary to neutralise the crabs prior to the fatal attack. Fewer non-fatal attacks prior to the fatal attack were committed on small crabs, which is likely due to them having smaller chelae and appearing less intimidating to potential predators. Alternatively, this trend may arise due to the relative cost benefit ratio with smaller crabs offering low energy returns for a similar handling time. A tethering study conducted with *C. maenas* in several Californian bays also found no effect of sex on the proportion of predated crabs, but there was a significant effect of size on survival with smaller crabs more likely to be preyed upon (De Rivera et al. 2005). The findings of the tethering experiments suggest that if *Carcinus* were to spread from its current distribution into natural habitat, there may be biotic resistance from predatory fish populations. This, in addition to the inability for *C. maenas* to withstand the high hydrodynamic forces (Hampton & Griffiths 2007) typical of the wave exposed Cape coastline, may explain why the species is restricted to Table Bay and Hout Bay harbours (see Chapter 2 for further details).

These findings may also offer insight into why this crab has to date not spread further along the South African coastline. All of the fish species that preyed upon *Carcinus* are found along the south coast of South Africa (Branch et al. 2010, De Vos et al. 2014, Roberson et al. 2015) and it is notable that the mean water temperatures in this region (Smit et al. 2013) are also appropriate for *C. maenas* survival and reproduction (Berril 1982, Behrens Yamada 2001). This suggests predator-driven biotic resistance may play a role in reducing the crab's impact or even preventing establishment. In Massachusetts, the abundance and distribution of *C. maenas* is limited through the predation by several native crab species including *Callinectes sapidus* (de Rivera et al. 2005) and *Cancer productus* (Hunt & Behrens Yamada 2003, Jensen et al. 2007). There are, however, no such crab species which could reduce the invasive potential of *Carcinus* through predation along the South African coast, but fish predators may well explain the restricted distribution of the crab. However, biotic resistance has not

been directly assessed in this study and should be considered in future research through dedicated field and laboratory experiments.

This study set out to experimentally test two hypotheses. The lack of evidence of impacts of *Carcinus* on harbour communities refuted the first of these, i.e. that as in other invaded regions this crab would cause measurable changes to the native biota. Based on these findings the ecological impacts of *Carcinus* would, under many impact classification schemes, including the EICAT scheme proposed by Blackburn et al. (2014), be categorised as having minor impact in South Africa. The second hypothesis that native predators such as fish and diving sea birds would consume this crab was only partly upheld, as no predators attacked *Carcinus* in a harbour environment but numerous fish species preyed upon the crab in an aquarium setting. While neither of these outcomes definitively reflects the potential response of predators to the crab in natural habitats, these results do demonstrate that fish are able to prey upon *Carcinus* and suggest that native predators may offer biotic resistance to the spread of this invasive crab along the open coast. Overall this study illustrates the potential risk of predicting impacts based on the impact history of a species elsewhere and highlights the need for locally relevant studies to support management decisions.

CHAPTER 5: UNDERSTANDING THE HISTORICAL AND CONTEMPORARY DYNAMICS OF *CARCINUS* IN SOUTH AFRICA

Abstract

Molecular approaches can be useful for inferring the colonisation history of invasive species in the absence of historical records. This is particularly insightful for species that have a wide global range and where understanding introduction dynamics will affect the choice of management approach. The European shore crab, *Carcinus maenas*, along with its congener, the Mediterranean shore crab, *C. aestuarii,* are two such species. *Carcinus* was first detected in South Africa in 1983 and was recently the target of a trial management programme in Hout Bay harbour. Attempting eradication from South Africa is only feasible if re-introduction can be prevented. The present study used microsatellites to assess the genetic diversity and structure of *Carcinus maenas* and *C. aestuarii* populations across selected native (England, Ireland, Portugal and Italy) and globally invasive (Australia, Canada, USA and Japan) ranges, including the only known South African populations (Hout Bay and Table Bay harbours). Population genetic data were used in Bayesian assignment tests and approximate Bayesian computations (ABC) to investigate the population genetic structure and invasion history of the genus in South Africa, respectively. To determine whether management was sufficiently severe to affect standing genetic diversity and structure of the *Carcinus* population in Hout Bay, I analysed samples collected prior to, during and after management. Table Bay harbour, which had undergone no management, was also sampled over the same time period. South African populations had higher genetic diversity than any of the other invasive *Carcinus* populations included here, suggesting that this introduction(s) was characterised by high propagule pressure. The most supported introduction scenario identified by the ABC analyses suggested that South African populations resulted from an admixture event between native and invasive *C. maenas* populations approximately 150 years ago. Evidence of interspecific hybridization between *C. maenas* and *C. aestuarii* was also supported, with the presence of several hybrid genotypes. Table Bay and Hout Bay harbours showed no genetic structure, suggesting migration had occurred between these two populations or that they are the result of the same introduction. Following management of *Carcinus* in Hout Bay harbour, there were no changes detected in the genetic diversity or structure of the population and its effective population size remained unchanged. Migration is unlikely to have occurred from Table Bay to the degree that no effect would be observed in the managed population if population size in the latter was dramatically decreased. The most likely explanation for this observation is that the next cohort of crabs, which were too small to be captured during management, grew to a size in 2016 that enabled capture during sampling or that management interventions did not reduce the population size to levels impacting standing genetic diversity. This, combined with the crabs remaining after unsuccessful management, maintained the gene frequencies of the previous generation, i.e. did not experience genetic drift due to low population size following management interventions. Given the potential

for migration, even at low levels, it can be concluded that managing a single *Carcinus* population in South Africa would likely prove ineffective. Moreover, the data suggest repeated introductions to South Africa have occurred in the past, implying that eradication would be futile unless introduction pathways are demonstrably closed.

Introduction

Understanding invasion histories in terms of pathways and propagule pressure, and post-introduction demographic processes of non-native species are important considerations for risk assessment and the development of effective management approaches (Drake & Lodge 2006, Johnston et al. 2009, Le Roux & Wieczorek 2009). For terrestrial organisms, these aspects are often easily quantified through historical introduction records (e.g. non-native trees introduced for forestry purposes (Richardson 1998)) and population level demographic studies (e.g. Zenni et al. 2009). When considering such processes in marine systems, there are unique challenges, including the fact that accidental introductions account for most marine invasions, resulting in introduction dynamics being difficult to infer (Hewitt & Campbell 2007, Mead et al. 2011a). In addition, many marine species have pelagic life stages in highly connected systems, potentially diluting pathway signatures (Johnston et al. 2009). Molecular approaches can, however, provide valuable proxies for these inferences as genetic diversity and its structure can be informative about historical and contemporary population level processes (Le Roux & Wieczorek 2009). Estimates such as the number of founding individuals, introduction frequency, population contractions and expansions, incidences of admixture and hybridization, can all be inferred from molecular data (Holland 2000, Rius et al. 2008, Geller et al. 2010, Rius et al. 2012, Rius & Darling 2014, Rius et al. 2015).

The European shore crab, *Carcinus maenas*, is one of the world's most widespread and successful marine invaders (Behrens Yamada 2001, Grosholz & Ruiz 2002, Carlton & Cohen 2003). Native to Europe, this species has established and become invasive on four continents: North America, South America, Australia and Africa (Carlton & Cohen 2003). Its congener, the Mediterranean shore crab, *C. aestuarii*, has also established populations outside of its native range in Japan (Sakai 1986). This broad non-native range suggests that the genus is easily transported over long distances, perhaps in its pelagic larval life stage (Queiroga 1996). Several studies have investigated the molecular ecology of *Carcinus* species across their invasive ranges (Bagley & Geller 2000; Geller et al. 1997; Roman & Palumbi 2004; Roman 2006, Darling et al. 2008, Tepolt et al. 2009). *Carcinus maenas* was first detected in South Africa in 1983 (Joska & Branch 1986). Previous genetic studies found mitochondrial haplotypes of both *C. maenas* and *C. aestuarii* in South Africa together with hybrid nuclear genotypes, suggesting the presence of both species and the occurrence of interspecific hybridisation (Geller et al. 1997, Darling et al. 2008). Evidence of hybridisation

was also detected in an invasive population of *C. aestuarii* in Japan (Darling 2011). It has been suggested that the Japanese population likely resulted from a single introduction event from a hybrid zone, where both *C. maenas* and *C. aestuarii* exist sympatrically (Darling 2011). However, the genetic analyses of the South African population were conducted over two decades ago and require a more in depth and updated assessment. The introduction history of these crabs to South Africa has not been assessed to date.

In South Africa, *Carcinus* is largely restricted to two harbours (Hout Bay and Table Bay, Figure 2.1). A recent study estimated the harbours contained approximately 7,000 and 164,000 individuals respectively (Jooste 2013). In order to assess the feasibility of a marine extirpation in South Africa, the crabs were targeted in a pilot management programme in Hout Bay harbour (see Chapter 3). However, it was discovered that the population size of *Carcinus* in Hout Bay (and most likely Table Bay) had been severely underestimated (see Chapter 3). A prerequisite for successful management of invasions is the prevention of future introductions of the target species (Myers et al. 2000). Therefore, in order to increase the probability of management success, information on the source of historical and contemporary introductions to South Africa is crucial.

Using a population genetic approach this study aimed to address the following questions: 1) Do South African populations of *Carcinus* have similar levels of genetic diversity and structure to globally invasive and native range populations?; 2) What are the most likely scenarios that explain the colonisation history of *Carcinus* in South Africa?; 3) Is hybridisation between *C. maenas* and *C. aestuarii* evident in South African populations?; 4) Do Table Bay and Hout Bay harbours represent independent introductions? 5) Have management efforts of *Carcinus* affected the genetic diversity, structure and effective population sizes of the Hout Bay population compared to the unmanaged Table Bay population?

Materials and Methods

Genetic material was collected from native and invasive populations of *Carcinus maenas* and *C. aestuarii* from around the world in 2015 (Table 5.1). South African samples were collected at two sites, Hout Bay and Table Bay, over three years (in 2014, 2015 and 2016). These temporal samples represent the population in Hout Bay before, during and after management of the crab (see Chapter 3). Table Bay was used as a control site, and sampled during the same period, where no management of the crab was conducted.

Table 5.1. The number of samples successfully analysed from populations in both the native and invasive ranges of *Carcinus maenas* and *C. aestuarii*.

Species	Population (abbreviation)	Native or invasive	Number of samples
C. maenas	Plymouth, England (ENG)	Native	49
C. maenas	Newtownards, Northern Ireland (IRE)	Native	49
C. maenas	Aveiro, Portugal (POR)	Native	48
C. maenas	Port Gawler, Australia (AUS)	Invasive	30
C. maenas	Stephenville, Canada (CAN)	Invasive	50
C. maenas	Bodega Bay, United States of America (USA)	Invasive	49
C. aestuarii	Chioggia, Italy (ITA)	Native	43
C. aestuarii	Tokyo Bay, Japan (JAP)	Invasive	30
Carcinus spp.	**South Africa (RSA)**	**Invasive**	**370 (total)**
	Hout Bay 2014 (HB_14)	Invasive	60
	Hout Bay 2015 (HB_15)	Invasive	65
	Hout Bay 2016 (HB_16)	Invasive	76
	Table Bay 2014 (TB_14)	Invasive	26
	Table Bay 2015 (TB_15)	Invasive	72
	Table Bay 2016 (TB_16)	Invasive	71

In South Africa, *Carcinus* individuals were collected using baited traps (Figure 2.4) containing 200 g of crushed sardines in a muslin bag to allow the scent to dissipate with a soak time of two hours. Crabs were euthanised by freezing at -20°C and frozen gill tissue removed for DNA extractions. International samples were provided as gill tissue, with the exception of samples from the USA, that were chelae tissue. All tissue samples were stored in 90% ethanol until DNA extraction.

DNA was extracted from all samples using a QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, California, USA, supplied by Whitehead Scientific, Cape Town, South Africa) according to the manufacturer's protocol, with a few exceptions, as follows. Following the addition of Buffer AW2, the solution was centrifuged for 5 minutes at 13,300 rpm. The DNeasy mini spin column was then removed from the collection tube and centrifuged for a further 3 minutes at 13,300 rpm in a clean collection tube to remove excess ethanol. Following the addition of Buffer AW2, the solution was centrifuged for 5 minutes at 13,300 rpm. The final elution step was modified by pipetting 50 µl Buffer AE onto the DNeasy membrane to increase the final DNA concentration. DNA quality and concentrations were determined using the Nanodrop spectrophotometer (Infinite 200 PRO NanoQuant, Tecan Group Ltd, Männedorf, Switzerland) and all samples diluted to a final concentration of 20 ng/µl.

Polymerase chain reaction (PCR) was used to test the amplification of 23 microsatellite primers previously developed for *C. maenas* and *C. aestuarii* (Tepolt et al. 2006, Marino et al. 2008) (Appendix 5.1). Multiplex kits were utilised in order to process the large number of

samples. The microsatellites were trialled under numerous enzyme and temperature conditions, however, successful amplification was only consistently achieved for eight loci (Table 5.2). PCR amplifications were performed using two multiplex reaction setups (QIAGEN Multiplex PCR kit and KAPA2G Fast Multiplex PCR kit supplied by Whitehead Scientific and Lasec, Cape Town, South Africa respectively). QIAGEN multiplex PCR reactions contained 1.5 µl x 0.2 µM primer mix (primers mixed in equal concentrations), 7.5 µl QIAGEN multiplex mix, 1.5 µl Q solution, 3 µl MilliQ water and 1.6 µl DNA. The KAPA2G Fast mutiplex PCR reactions contained 4.5 µl MilliQ water, 7.5 µl 2x Kapa2G Fast Multiplex mix, 1.5 µl x 2 µM primer mix, and 1.6 µl template DNA. PCR reactions were performed in 96 well plates (with nine replicate samples and two water samples per plate as controls) in a Labnet MultiGene Optimax thermal cycler (Labnet International, Inc., Edison, New Jersey, USA). PCR cycling conditions for the QIAGEN PCR reaction followed a 15 min initial activation step at 95°C, followed by 30 cycles of 30 s denaturation at 94°C, 90 s annealing at 52.7°C and extension for 60 s at 72°C, before a final extension for 30 min at 60°C. The KAPA2G Fast PCR protocol involved a 3 min initial denaturation step at 95°C, followed by 30 cycles of 15 s denaturation at 95°C, 30 s annealing at 54.4°C and 25 s extension at 72°C and then a final extension of 1 min at 72°C. Purified PCR fragments were separated on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA), using GENESCAN TM-500 (-250) as an internal size standard (Applied Biosystems). Allele sizes were visualized and scored using GENEMARKER v1.95 (SoftGenetics LLC, State College, Pennsylvania, USA). Samples that failed to amplify at more than four loci were excluded from the final dataset, with a total of 718 individuals remaining. For further details of sample sizes per population, see Table 5.1.

Table 5.2. Details of the eight successfully amplified microsatellite loci.

Multiplex in current study	Locus name	Species developed for	Original study
QIAGEN	Cma14EPA	*C. maenas*	Tepolt et al. 2006
	Cma04EPA	*C. maenas*	Tepolt et al. 2006
	Cae71	*C. aestuarii*	Marino et al. 2008
Kappa	Cae33	*C. aestuarii*	Marino et al. 2008
	Cae30	*C. aestuarii*	Marino et al. 2008
	Cae86	*C. aestuarii*	Marino et al. 2008
	Cma03EPA	*C. maenas*	Tepolt et al. 2006
	Cma02EPA	*C. maenas*	Tepolt et al. 2006

Global population structure

The final dataset was assessed for the presence of null alleles using the FreeNa software (Chapuis & Estoup 2007). Pairwise Fixation indices (F_{ST}) were calculated in FreeNa with and without the 'excluding null alleles' (ENA) correction factor to account for the presence of null alleles (Chapuis & Estoup 2007). In order to compute 95% confidence intervals for F_{ST} values, 10,000 simulations were performed. F_{ST} values for each population were then used

to test whether correction factors (i.e. the presence of null alleles) significantly influenced the results using a Kruskal-Wallis test.

The genetic structure of all sampled populations was estimated with a Bayesian approach in STRUCTURE version 2.3.4 (Pritchard et al. 2000). STRUCTURE uses Bayesian Monte Carlo Markov Chain (MCMC) sampling to identify the optimal number of genetic clusters for a given dataset by reducing departures from Hardy–Weinberg and linkage equilibrium expectations within genetic clusters. Models were run on the following datasets: 1) All global populations of *Carcinus*, including the South African populations (n = 14); and 2) All global populations of *Carcinus*, excluding all South African populations (n = 8). The range of *K* tested depended on the analysis, with *K* = 1-14 for the global analysis, *K* = 1-8 for the global analysis excluding South Africa. Twenty independent models were run for each value of *K*. Each model consisted of 500,000 generations of which the first 100,000 were discarded as burnin. The admixture model was applied with correlated allele frequencies as hybrid individuals (*C. meanas* X *C. aestuarii*) were suspected to be present in the dataset based on previous reports (Darling et al. 2008). The optimal *K* value was determined using the method described by Evanno et al. (2005) and STRUCTURE Harvester (Earl & von Holdt 2012). The programme CLUMPP (version 1.1.2; Jakobsson & Rosenberg 2007) was used to merge the results from all the STRUCTURE runs, utilising the greedy option method with 10,000 repeats and the resultant output file was used in DISTRUCT (version 1.1; Rosenberg 2004) to create bar plots.

Reconstructing the history of Carcinus introductions to South Africa

The introduction history of *Carcinus* into South Africa was inferred using an approximate Bayesian computation (ABC) approach and the programme DIYABC version 2.1.0 (Cornuet et al. 2008, Cornuet et al. 2014). This approach involves the generation of simulated demographic datasets (including source populations, genetic bottlenecks, etc.) that are compared to the observed data using a specified set of summary statistics to determine similarity and the posterior distributions of the model parameters.

Analysis 1 involved a broad set of simulations that considered all potential sources of *Carcinus*. The probabilities of five invasion scenarios were estimated, which in this case, included an introduction from any of the six *C. maenas* and two *C. aestuarii* populations sampled, as well as the potential for the source to be a ghost, i.e. unsampled, population (Figure 5.1.) The entire global dataset was used for this analysis, excluding the 2014 and 2015 South African samples.

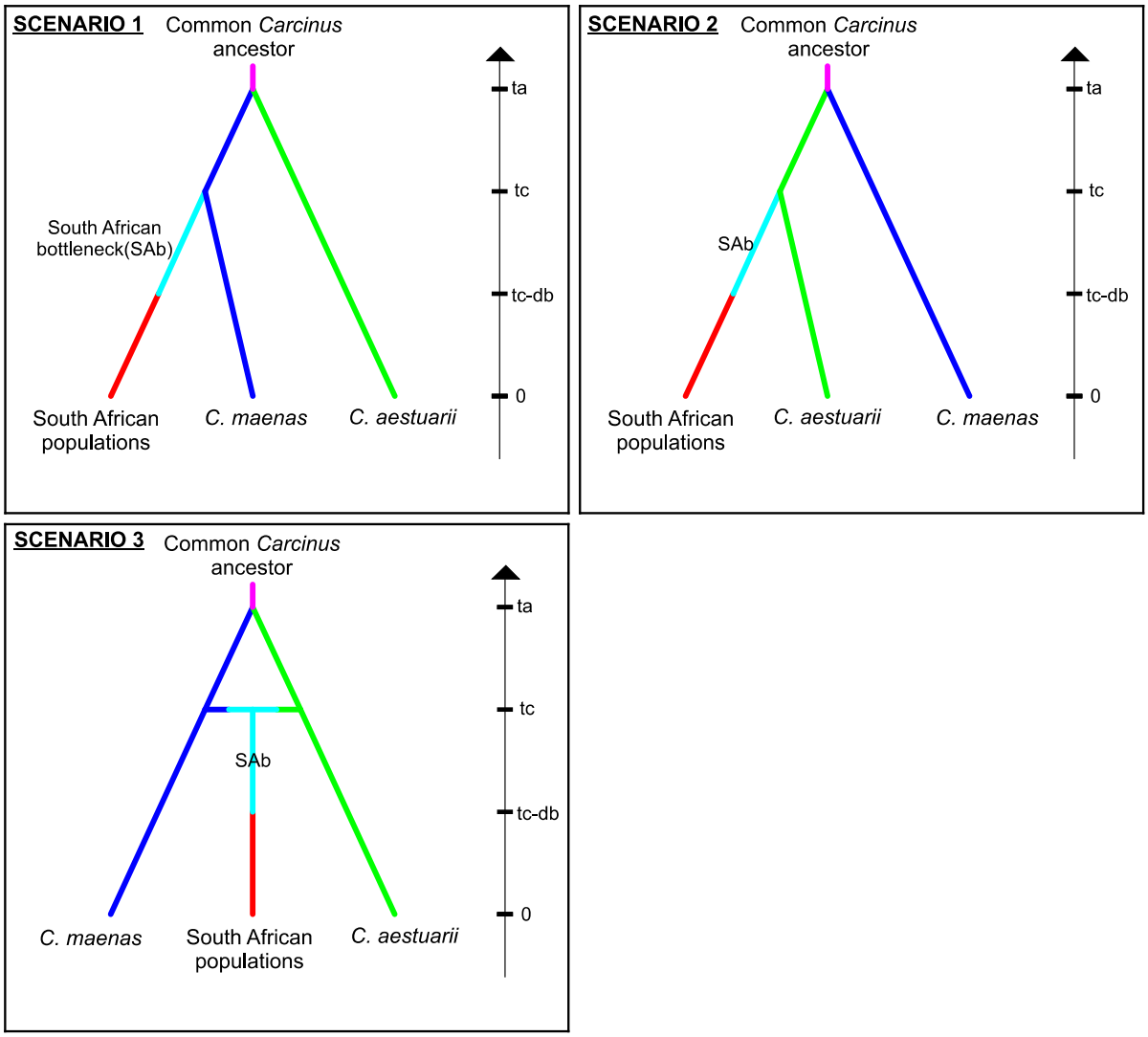

Figure 5.1. Potential invasion scenarios for *Carcinus* introductions into South Africa (Analysis 1). Scenario descriptions as follows: Scenario 1) Direct introductions from the native or invasive range of *C. maenas* to South Africa, with a genetic bottleneck; Scenario 2) As Scenario 1, but a direct introduction from the native or invasive range of *C. aestuarii*; Scenario 3) An admixture event between *C. maenas* and *C. aestuarii* leads to the founding of the South African population after a bottleneck. Note that time is not to scale.

Analysis 2 was conducted at a finer resolution, with the scenarios developed using the inferred results of the previous model, and led to a more detailed investigation of the source(s) of South African populations (Figure 5.2). The results of Analysis 1 indicated that *C. maenas* was most likely the species that was responsible for the South African invasion, and therefore *C. aestuarii* was excluded from this second analysis. This allowed the role of native and invasive *C. maenas* populations to be explored in greater detail to determine the likely ancestral population. This analysis utilised the *C. maenas* dataset (including populations in the native and invasive ranges), with the South African populations sampled in 2016 only.

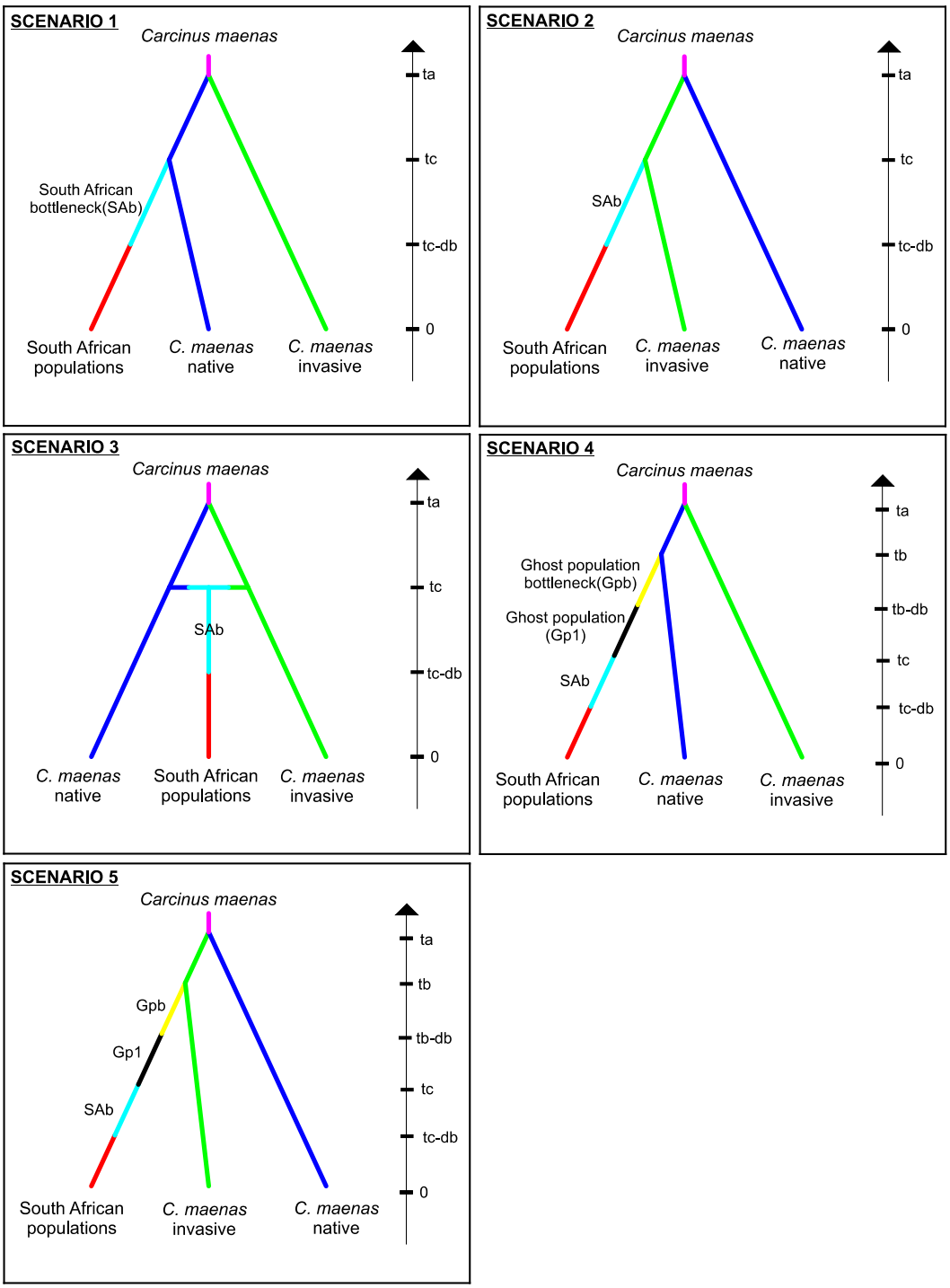

Figure 5.2. Potential invasion scenarios for *Carcinus* in South Africa (Analysis 2). Scenario descriptions as follows: Scenario 1) Direct introductions from the native range of *C. maenas* to South Africa, with a genetic bottleneck; Scenario 2) Direct introductions from the invasive range of *C. maenas* to South Africa, with a genetic bottleneck; Scenario 3) An admixture event between the native and globally invasive *C. maenas* leads to the founding of the South African population after a bottleneck; Scenario 4) A ghost population in the *C. maenas* native range founds the South African population (with each population experiencing a bottleneck); and Scenario 5) A ghost population in the *C. maenas* invasive range founds the South African population (with two bottlenecks). Note that time is not to scale.

The models were parameterised by drawing on the species' biology and observed invasion events. It was assumed that the two congeners shared demographic parameters where data were unavailable for *C. aestuarii* (the least studied of the two species). The timing of events was measured in terms of generations. In the case of *Carcinus*, each generation time was considered to be one year, based on the minimum previous inferences for *C. maenas* (Behrens Yamada 2001). The values estimated for the temporal priors were assumed from historical shipping and detection records (Joska & Branch 1986, Mead et al. 2011a). The prior parameters (estimated with uniform distributions) for temporal events, population sizes and bottleneck reductions are detailed in Table 5.3. Loci were estimated to have an allelic range between 40 and 72. The minimum value of 40 represented the DIYABC default value, which is greater than recorded in this study, but allowed for the inclusion of alleles that were potentially unsampled in this study. The Single Step Mutation model was selected over the default generalised stepwise mutation model offered in DIYABC. Microsatellite mutation rates have not been determined for crustaceans, however for mammals, rates are between 1×10^{-3} to 1×10^{-5} (Dallas 1992, Ellegren 1995) while in other arthropods (e.g. *Drosophila melanogaster*), mutation rates of 6×10^{-6} have been observed (Schlötterer et al. 1998). Accordingly, rates in this study ranged from 1×10^{-2} to 1×10^{-6}. The DIYABC default rates of admixture were used, i.e. 0.001-0.999. The summary statistics used to differentiate between the different scenarios modelled included the mean number of alleles and the mean genic diversity (Nei 1987) (as measures of the diversity among the sampled populations), in addition to the fixation index (F_{ST}) (Weir & Cockerham 1984) and genetic distance $(d\mu)^2$ (Goldstein et al. 1995) to distinguish between populations. Datasets were simulated 1×10^6 times for each scenario. The pre-evaluation analysis allowed a visual comparison between the simulated and observed datasets of each scenario in a principal co-ordinate analysis (PCA) as well as a ranked comparison between the summary statistics of each dataset. The posterior probabilities were then compared in a Linear Discriminant Analysis using a logistic regression approach to select the 1% closest simulated dataset to that observed, with non-overlapping 95% confidence intervals. The accuracy of the posterior distributions of the model parameters were evaluated using the relative bias. In order to evaluate the confidence in scenario choice, various scenario-parameter combinations were analysed in the prior and posterior distributions. The so-called Type I and II errors were calculated using 100 pseudo-observed datasets (pods) for each scenario. For analysis 1, type I errors were estimated by counting the number of times that Scenario 1 was not selected, over the 100 pods. Type II errors were calculated based on the proportion of times Scenario 1 was selected when it did not have the highest posterior probability. The type I errors for analysis 2 were estimated based on the proportion of times Scenario 3 was not selected, while type II errors were calculated using the proportion of Scenario 3 false acceptances.

Table 5.3. Prior parameters selected for the two Bayesian analyses using historical records. The numbering refers to previous studies used to populate the model parameters: [1] Joska & Branch 1986; [2] Mead et al. 2011b. Analysis 1 was conducted on the global dataset (all *C. maenas* and *C. aestuarii* populations and the 2016 South African populations), while analysis 2 investigated the probability of the South African populations arising from *C. maenas* populations in the native or invasive ranges. The units for population sizes are the numbers of individuals and time is measured in generations (years).

Analysis	Parameter	Description	Min. value	Max. value
1	Na; N1-N3	Initial size of all populations	100	10,000
	ta	*C. maenas* and *C. aestuarii* divergence	10	10,000
	tb	Formation of ghost population [1]	33	400
	tc	Introduction of South African population [1,2]	33	400
	db	South African bottleneck [1]	5	33
	SAb	Reduced size of South African population	5	1,000
2	Na; N1-N5	Initial size of all populations	100	100,000
	ta	Divergence of *C. maenas* native and invasive populations [1,2]	33	100,000
	tb	Formation of ghost population [1]	33	100,000
	tc	Introduction of South African population [1,2]	33	364
	db	South African bottleneck [1]	5	33
	SAb	Reduced size of South African population	5	1,000
	Gpb1-2	Reduced size of ghost populations	5	1,000

Comparative regional genetic diversity

The number of alleles per locus were calculated using the 'NB' package in the R statistical environment (R core team, 2016). Observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (A_R), and the inbreeding coefficient (F_{IS}), and significant departures of allele frequencies from Hardy Weinberg equilibrium (HWE) expectations were calculated in the 'diveRsity' (Keenan et al. 2013) package in R.

The genetic diversity metrics were compared across regions for the *C. maenas* populations. Estimates for A_R, H_O and F_{IS} were examined with respect to region (3 levels: invasive global, invasive South African and native populations) using ANOVAs. Expected heterozygosity was examined using a Kruskal-Wallis test as the data were not normally distributed. Tukey's posthoc tests or non-parametric multiple comparison tests were performed following the detection of significant effects.

Genetic demographic impacts of management interventions against Carcinus *in South Africa*

Allelic richness of the three sampling periods in Table Bay and Hout Bay harbours before (2014), during (2015) and after (2016) management interventions in Hout Bay (see Chapter 3) was calculated using the 'diveRsity' (Keenan et al. 2013) package in R (R core team, 2016). The A_R was analysed with respect to harbour (2 levels: Table Bay and Hout Bay) and year (3 levels: 2014, 2015 and 2016), using a two-factor generalized linear model (GLM) with a quassipoisson error distribution. The number of P_A and the N_e were analysed with respect to harbour and year using a Pearson's Chi Squared test.

Temporal changes in the genetic structure of South African populations was estimated using Bayesian assignment tests in STRUCTURE (version 2.3.4; Pritchard et al. 2000). This methodology was identical to that of the global population structure analysis, except that a range of K = 1-6 was tested for the South African-only analysis. The assignment values for the South African populations were analysed with respect to harbour (2 levels: Table Bay and Hout Bay) and year (3 levels: 2014, 2015 and 2016), using a two-factor generalized linear model (GLM) with a quassipoisson error distribution.

Estimates of contemporary effective population sizes (N_e) are useful in conservation and the management of invasive species as the metric can be an indicator of population viability and eradication potential (Zalewski et al. 2016). This differs from the DIYABC estimate of N_e which measures long term effective population sizes. Estimates of contemporary N_e of each harbour was estimated for each sampling period independently, using the single sample Linkage Disequilibrium method in NeEstimator version 2.0.1 (Do et al. 2014). A criterion for excluding rare alleles (P_{crit}) was provided to reduce bias in the population estimates, as the presence of rare alleles is demonstrated to overestimate the N_e (Waples & Do 2010). In this study, P_{crit} values of 0.02 (excluding alleles with a frequency less than 2%) were considered, as recommended by Waples & Do (2010) when sample sizes are greater than 25. Pseudo jackknife resampling methods were used to estimate the confidence intervals (*sensu* Jones et al. 2016).

Results

Global population structure

Null alleles were detected in the dataset but at a low frequency, with a mean of 0.036 (SD: 0.056) across all loci. Seven of the microsatellite loci were identified as polymorphic in all populations included, with a maximum of 36 alleles detected per locus. One locus was monomorphic in 6 of the 14 populations, but had a total of five alleles across the global range. Thirty-one (28%) loci by population combinations showed significant departures (p < 0.05) from HWE in the 14 populations.

There was no significant difference between pairwise F_{ST} values when uncorrected or corrected for the presence of null alleles (Table 5.4 and 5.5 respectively, H = 0.394, p = 0.530), therefore subsequent analyses were conducted using the uncorrected dataset. Among global *Carcinus* populations (i.e. excluding South Africa), pairwise F_{ST} distances ranged from zero between Portugal and Ireland, to 0.2473 between the USA and Japan (Table 5.4). Not surprisingly, the highest differentiation was observed between *C. maenas* and *C. aestuarii* populations. STRUCTURE analyses revealed four optimal number of genetic clusters (K = 4) within the global range of the genus *Carcinus* (Figure 5.3a, Figure 5.3b). These consisted of a distinct *C. aestuarii* cluster (including native Italian and invasive Japanese populations), a North American cluster (comprised of the USA and Canadian populations), a *C. maenas* cluster (including English, Irish, Portugese and South African populations) and a less distinct cluster, that corresponded mostly to Australia. When considering the global range of *Carcinus* excluding South Africa, the optimal number of genetic clusters identified was three (K = 3) (Figure 5.3c, Figure 5.3d). Here, Australia was assigned to the same cluster as native *C. maenas*, while the *C. aestuarii* and North American populations comprised two distinct clusters.

Table 5.4. Pairwise F_{ST} values calculated between all sampled *Carcinus* populations, without ENA correction (Chapuis & Estoup 2007). All pairwise comparisons were non-significant ($p > 0.05$).

Population	ENG	IRE	ITA	POR	AUS	CAN	HB_14	HB_15	HB_16	JAP	TB_14	TB_15	TB_16
IRE	0.0001	-	-	-	-	-	-	-	-	-	-	-	-
ITA	0.1045	0.0934	-	-	-	-	-	-	-	-	-	-	-
POR	0.0030	0.0000	0.0949	-	-	-	-	-	-	-	-	-	-
AUS	0.0585	0.0539	0.1153	0.0570	-	-	-	-	-	-	-	-	-
CAN	0.0294	0.0324	0.1319	0.0369	0.0885	-	-	-	-	-	-	-	-
HB_14	0.0039	0.0057	0.1083	0.0050	0.0627	0.0374	-	-	-	-	-	-	-
HB_15	0.0044	0.0084	0.1002	0.0062	0.0614	0.0372	0.0019	-	-	-	-	-	-
HB_16	0.0127	0.0155	0.0935	0.0131	0.0768	0.0431	0.0106	0.0111	-	-	-	-	-
JAP	0.1525	0.1498	0.1386	0.1539	0.1998	0.1967	0.1611	0.1624	0.1572	-	-	-	-
TB_14	0.0004	0.0056	0.0885	0.0023	0.0600	0.0350	0.0000	0.0000	0.0000	0.1493	-	-	-
TB_15	0.0053	0.0061	0.0895	0.0024	0.0604	0.0393	0.0004	0.0007	0.0081	0.1474	0.0000	-	-
TB_16	0.0051	0.0077	0.1049	0.0040	0.0715	0.0392	0.0006	0.0015	0.0037	0.1588	0.0000	0.0000	-
USA	0.1012	0.1020	0.1666	0.1006	0.1419	0.0677	0.1015	0.1043	0.1078	0.2473	0.0993	0.0992	0.1036

Table 5.5. Pairwise F_{ST} values calculated between all sampled *Carcinus* populations, with ENA correction (Chapuis & Estoup 2007). All pairwise comparisons were non-significant ($p > 0.05$).

Population	ENG	IRE	ITA	POR	AUS	CAN	HB_14	HB_15	HB_16	JAP	TB_14	TB_15	TB_16
IRE	0.0004	-	-	-	-	-	-	-	-	-	-	-	-
ITA	0.1022	0.0932	-	-	-	-	-	-	-	-	-	-	-
POR	0.0033	0.0003	0.0973	-	-	-	-	-	-	-	-	-	-
AUS	0.0534	0.0482	0.1133	0.0526	-	-	-	-	-	-	-	-	-
CAN	0.0288	0.0318	0.1330	0.0357	0.0812	-	-	-	-	-	-	-	-
HB_14	0.0062	0.0080	0.1080	0.0064	0.0581	0.0378	-	-	-	-	-	-	-
HB_15	0.0050	0.0090	0.1012	0.0057	0.0565	0.0358	0.0030	-	-	-	-	-	-
HB_16	0.0091	0.0123	0.0930	0.0090	0.0669	0.0374	0.0076	0.0072	-	-	-	-	-
JAP	0.1444	0.1420	0.1317	0.1451	0.1912	0.1877	0.1472	0.1529	0.1482	-	-	-	-
TB_14	0.0013	0.0041	0.0923	0.0005	0.0542	0.0321	0.0000	0.0000	0.0000	0.1461	-	-	-
TB_15	0.0057	0.0071	0.0905	0.0024	0.0552	0.0383	0.0016	0.0004	0.0047	0.1387	0.0000	-	-
TB_16	0.0064	0.0094	0.1064	0.0044	0.0670	0.0383	0.0022	0.0013	0.0013	0.1505	0.0000	0.0000	-
USA	0.0997	0.1017	0.1672	0.1018	0.1351	0.0662	0.1014	0.1031	0.1041	0.2395	0.1005	0.0987	0.1037

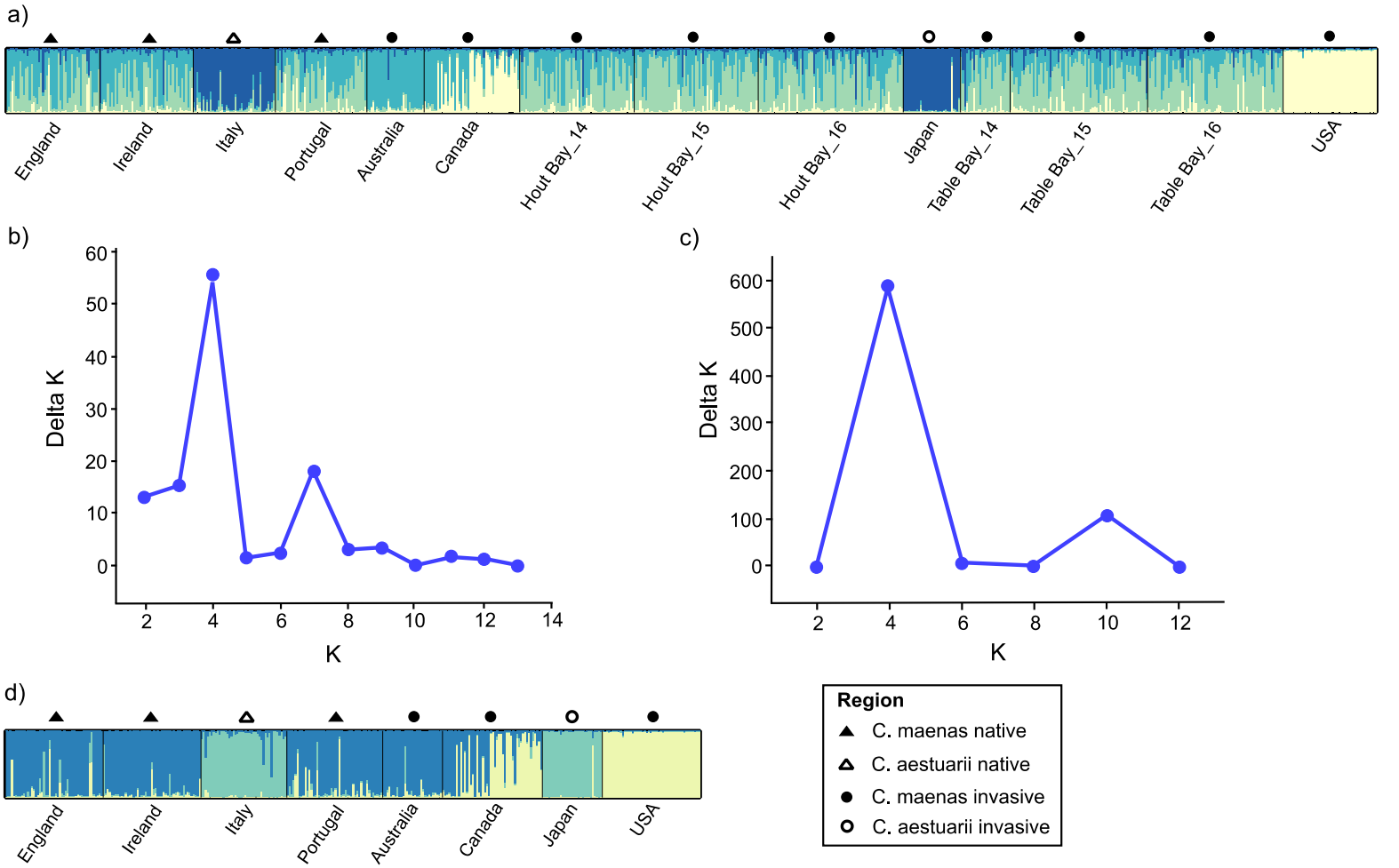

Figure 5.3. STRUCTURE bar plots where vertical axes illustrate the proportional assignment of individual genomes to the inferred genetic clusters where a) *K* = 4 for global *Carcinus* populations sampled, including both native and invasive regions; b) the Delta *K* plot for the global dataset showing the optimal number of ancestral clusters; c) the Delta *K* plot for the global dataset excluding the South African populations; and d) *K* = 3 for all global populations sampled excluding South African populations.

Reconstructing the history of Carcinus *introductions to South Africa*

The most probable invasion scenario was that a *C. maenas* population founded the South African population i.e. Scenario 1 (Figure 5.4a, Figure 5.4b). This had the highest posterior probability with 95% confidence intervals that did not overlap with the other two scenarios (P = 0.5120, 95%CI = 0.5028 – 0.5213). The type I error associated with the selection of this scenario was 0.05 using the direct approach and 0.09 for the logistic approach. The mean type II errors calculated under the direct and logistic approach were 0.12 (ranging from 0 to 0.24) and 0.08 (with a range of 0 to 0.16) respectively. The next most likely scenario inferred by the model was that admixture between *C. maenas* and *C. aestuarii* resulted in the South African invasion, i.e. Scenario 3 (P = 0.4731, 95%CI = 0.4638 – 0.4824). The last scenario (which inferred that the colonising propagules came from a *C. aestuarii* population) was not well supported by the model (P = 0.0148, 95%CI = 0.0066 – 0.0231).

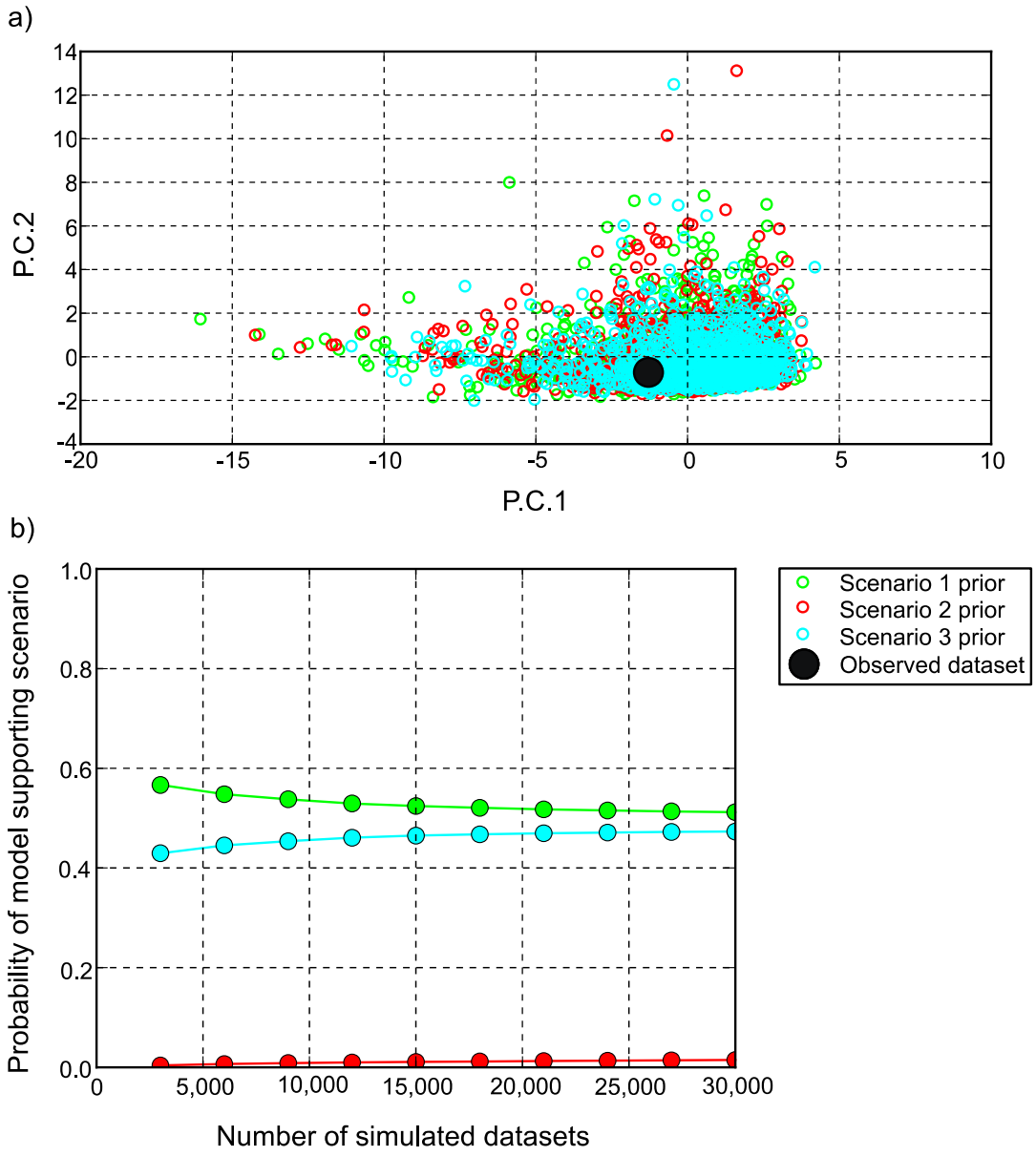

Figure 5.4. a) Principal Co-ordinate Analysis (PCA) showing the similarity between the observed microsatellite data set and the three modelled scenarios inferring the invasion of *Carcinus* to South Africa from populations of *C. maenas* and/or *C. aestuarii;* b) A comparison of the posterior probabilities from the Linear Discriminant Analysis using a logistic regression approach. In this analysis, scenario 1 (an introduction to South Africa from the *C. maenas* range) is the most parsimonious.

The second analysis convincingly showed that the most supported scenario involved an admixture event between sampled populations in the native and invasive range (i.e. Scenario 3) (P = 0.758, 95%CI = 0.744 – 0.771) (Figure 5.5a, Figure 5.5b). Type I errors associated with the selection of Scenario 3 were 0.17 using the direct approach and 0.15 for the logistic approach. The mean type II errors associated with the false acceptance of Scenario 3 under the direct and logistic approach were 0.03 (ranging from 0.01 to 0.05) and 0.05 (with a range from 0.03 to 0.06) respectively. The next most probable explanation for the genetic patterns observed in South Africa inferred that the colonists came from a population in the *C. maenas* native range (P = 0.173, 95%CI = 0.160 – 0.186).

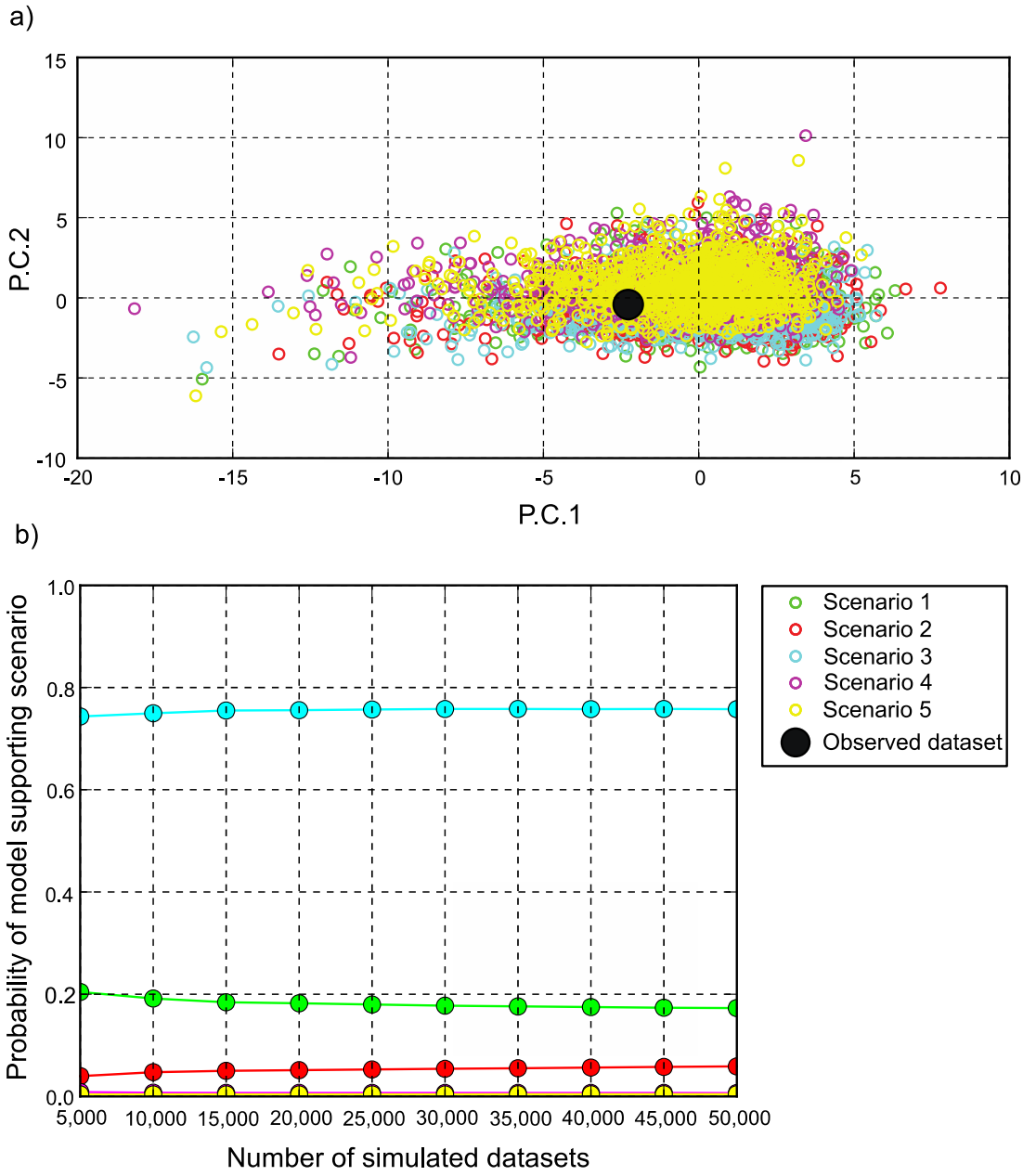

Figure 5.5. a) Principal Co-ordinate Analysis (PCA) showing the similarity between the observed microsatellite data set and the five modelled scenarios inferring the invasion of *Carcinus* to South Africa from the native and invasive populations of *C. maenas*; b) A comparison of the posterior probabilities from the Linear Discriminant Analysis using a logistic regression approach. This analysis inferred that scenario 3 (i.e. *Carcinus* was introduced to South Africa following an admixture event between *C. maenas* native and invasive populations) is the most likely.

The effective size of the South African population according to the posterior distributions of the Bayesian model parameters in analysis 1 was 5,410 (95%CI: 1,490

– 9,710) while analysis 2 estimated the populations to comprise 7,100 individuals (95% CI: 2,440 – 9,910) (Table 5.6). The optimal invasion scenario in analysis 1 estimated that *Carcinus* arrived in South Africa 155 (95% CI: 41-365) years ago, i.e. between 1651 and 1975. Analysis 2 estimated that the crab was introduced 143 (95%CI: 37-338) years ago, i.e. 1678 to 1980. Both analyses estimated that there was a genetic bottleneck that affected the population for 17 generations (Analysis 1 95%CI: 5-32; Analysis 2 95% CI: 6-31). As a result, effective number of founder individuals was estimated by analysis 1 to be 517 (95% CI: 104-971), while analysis 2 estimated a founding population size of 598 (95% CI: 105-970).

Table 5.6. Posterior distributions of model parameters for the two ABC analyses conducted to infer the invasion history of *Carcinus* in South Africa. The distributions shown are those estimated by the most parsimonious scenario. For analysis 1, the most likely scenario was the introduction of *Carcinus* to South Africa from the *C. maenas* range (Scenario 1). For analysis 2, the scenario with the higher probability involved the introduction of *Carcinus* following an admixture event between populations in the *C. maenas* native and invasive ranges (Scenario 3).

Analysis	Parameter	Description	Units	Mean	Median	Mode	Lower CI	Upper CI
(1) *C. maenas* -> SAb -> South Africa	Na	Effective size of *Carcinus* prior to divergence	No. of crabs	4,860	4,770	4,910	354	9,690
	N1	Effective size of *C. maenas* population	No. of crabs	6,340	6,430	6,760	2,730	9,620
	N2	Effective size of *C. aestuarii* population	No. of crabs	5,380	5,250	5,150	1,710	9,510
	N3	Effective size of South African populations	No. of crabs	5,410	5,190	3,780	1,490	9,710
	ta	Divergence of *C. maenas* and *C. aestuarii*	Generations(years)	4,300	3,890	2,360	789	9,410
	tc	Introduction of South African population	Generations(years)	155	134	78	41	365
	db	Length of South African bottleneck	Generations(years)	17	17	6	5	32
	SAb	Reduced size of South African population	No. of crabs	517	497	374	104	971
(2) *C. maenas* native & invasive -> SAb -> South Africa	Na	Effective size of *C. maenas* population	No. of crabs	5,520	5,450	4,420	1,220	9,730
	N1	Effective size of *C. maenas* native population	No. of crabs	6,710	6,880	7,670	2,630	9,790
	N2	Effective size of *C. maenas* invasive population	No. of crabs	1,220	870	455	252	4,810
	N3	Effective size of South African populations	No. of crabs	7,110	7,520	9,890	2,440	9,910
	ta	Divergence of *C. maenas* native & invasive populations	Generations(years)	225	108	55	26	1,160
	tc	Introduction of South African population	Generations(years)	143	122	44	37	338
	db	Length of South African bottleneck	Generations(years)	17	17	6	6	31
	SAb	Reduced size of South African population	No. of crabs	598	623	995	105	970

Comparative regional genetic diversity

All native populations demonstrated high A_R (10.835 to 11.993, Table 5.7). In comparison, the A_R of invasive populations ranged from 4.097 to 7.253, with the exception of South Africa where A_R ranged between 9.759 and 9.972. In all populations, the H_O was lower than H_E (Table 5.7). The H_O in native populations ranged from 0.655 to 0.671, while invasive populations ranged from 0.437 to 0.646. The P_A in the native range varied from 4 to 15, while the invasive ranges demonstrated lower numbers (less than 3 per population). The mean P_A detected in the combined South African populations was 9. F_{IS} estimates were higher in invasive, compared to native populations (Table 5.7), with Japan demonstrating the highest level of inbreeding at 0.26.

Table 5.7. Basic diversity statistics for the four native and five invasive populations, calculated as mean values of each index over the eight loci analysed.

Population	Species	Status	Allelic richness (A_R)	Observed heterozygosity (H_o)	Expected heterozygosity (H_E)	Private alleles (P_A)	Inbreeding coefficient (F_{IS})
ENG	*C. maenas*	Native	10.884	0.665	0.696	7	0.059
IRE	*C. maenas*	Native	10.835	0.671	0.706	4	0.044
ITA	*C. aestuarii*	Native	11.993	0.655	0.745	15	0.098
POR	*C. maenas*	Native	11.345	0.653	0.704	7	0.052
AUS	*C. maenas*	Invasive	7.113	0.584	0.654	1	0.106
CAN	*C. maenas*	Invasive	7.253	0.600	0.654	0	0.079
JAP	*C. aestuarii*	Invasive	4.097	0.437	0.544	3	0.260
RSA (mean)	*Carcinus*	Invasive	**10.31**	**0.628**	**0.694**	**9**	**0.187**
HB			9.759	0.646	0.686	3	0.164
TB			9.972	0.581	0.681	1	0.12
USA	*C. maenas*	Invasive	4.47	0.587	0.592	0	0.005

When comparing the regional differences in genetic diversity of *C. maenas* populations, there was no effect of region on the H_O (F = 0.346, df = 2, p = 0.708) (Figure 5.6a) or the estimated inbreeding (F = 0.212, df = 2, p = 0.809) (Figure 5.6b). However, significant differences in *C. maenas* A_R were recorded in different regions (F = 7.366, df = 2, p = 0.001), with the invasive global populations demonstrating lower A_R than both the native global and South African populations (Tukey's test, $p < 0.05$) (Figure 5.6c). Region also had a significant effect on the H_E of the crab, with the native and South African populations demonstrating higher H_E than the global invasive populations (H = 11.214, df = 2, p = 0.004; multiple comparisons, $p < 0.05$) (Figure 5.6d).

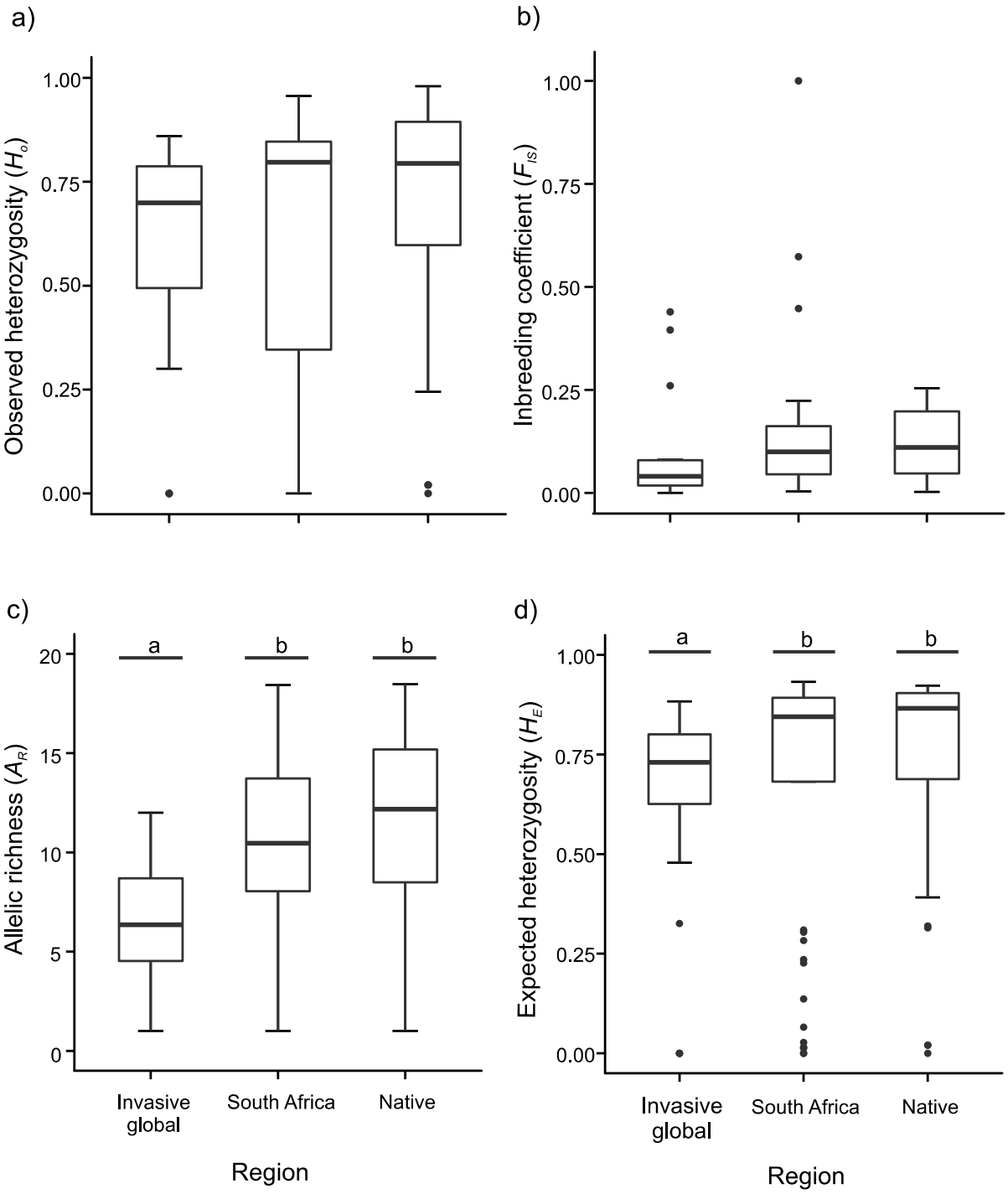

Figure 5.6. Comparative genetic diversity metrics a) H_O; b) F_{IS}; c) A_R; and d) H_E between the *Carcinus maenas* regions globally (invasive global comprised of Australia, Canada and USA; invasive South Africa, specifically Hout Bay and Table Bay; and native including England, Ireland and Portugal). Boxplots depict the median value, interquartile ranges and outliers of each region. The lettering refers to the main effect of region with groups sharing lettering not significantly different from each other ($p > 0.05$).

Within the South African populations, there was no main effect of harbour or year (or an interaction between the two) on A_R (Table 5.8, Figure 5.7.a). The A_R of the Hout Bay population did not change through time. There was a higher number of P_A in the Hout Bay population compared to Table Bay (X^2 = 14, df = 5, p = 0.0156) but this did not change over time (X^2 = 6, df = 6, p = 0.423, (Figure 5.7.b)).

Table 5.8. GLM results considering the effect of harbour (2 levels and year (3 levels) on A_R. See Appendix 5.2 for further details regarding the GLM output. All effects were non-significant (p > 0.05).

Factor	df	F	*p*
Harbour	1	0.0287	0.8663
Year	2	0.2162	0.8064
Harbour x Year	2	0.03329	0.9677

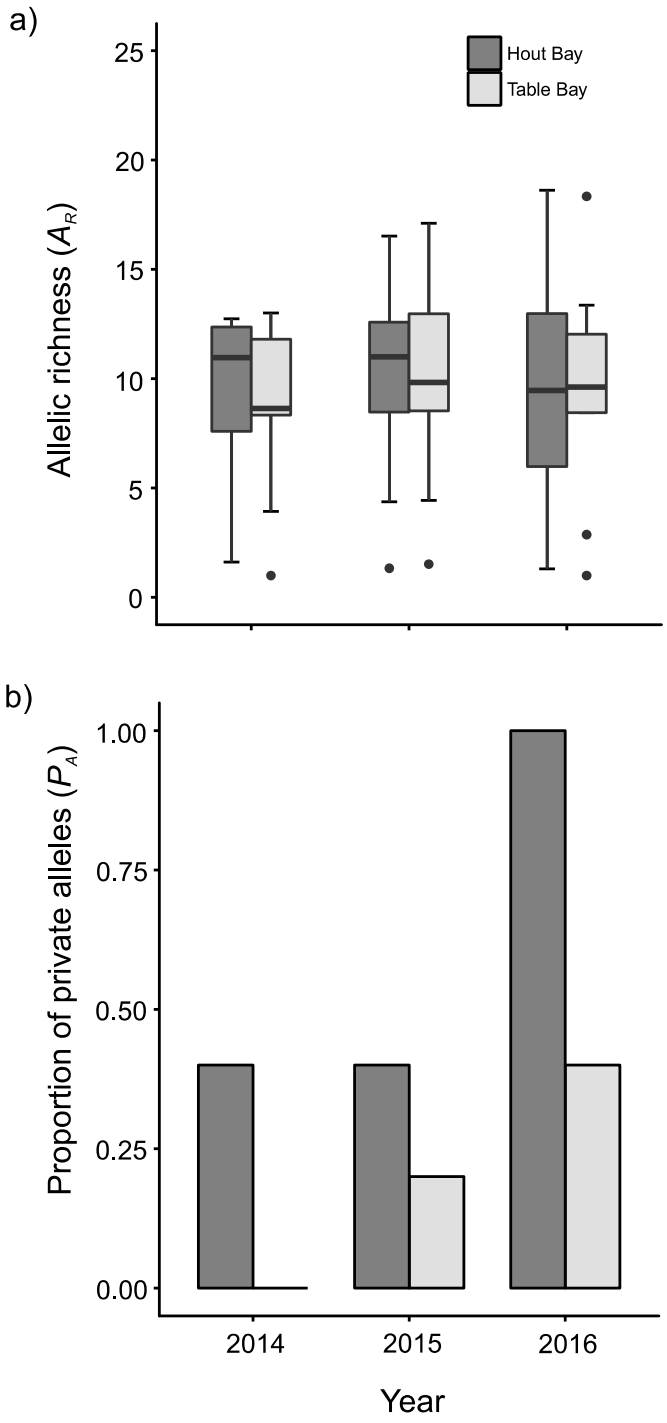

Figure 5.7. Temporal changes in a) A_R and b) the proportion of P_A observed in the South African *Carcinus* populations. All effects were non-significant ($p > 0.05$).

Among South African populations, the genetic differentiation was extremely low, ranging from 0 to 0.0076. When analysing only South African populations STRUCTURE assigned individuals to three genetic clusters (K=3), but with all individuals assigned with equal

proportions to all genetic clusters (Figure 5.8). Such complete and equal admixture indicates the true value of *K* is likely to be one (Le Roux et al. 2008).

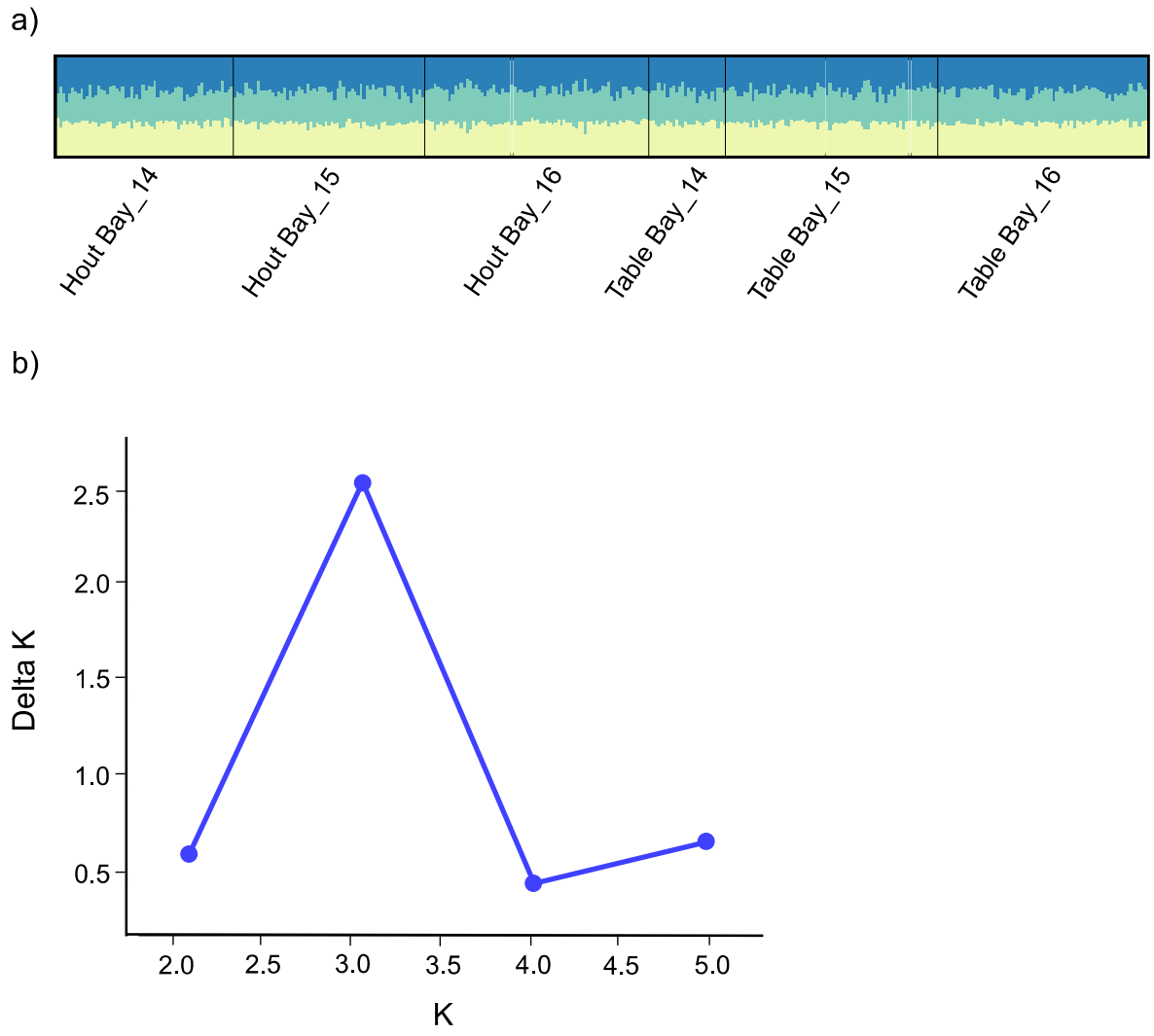

Figure 5.8. a) The structure of the South African *Carcinus* populations (*K* = 3); b) The Delta *K* plot for the South African populations showing the optimal number of ancestral clusters.

There was no main effect of year or site on the Bayesian assignment values for South African populations (q1-4) (Table 5.9, Figure 5.9a-d) when considering data from the full global model. None of the interactions between the two factors were significant, except for q2, where there was a significant interaction between site and year (F = 5.559, df = 2, *p* = 0.004).

Table 5.9. GLM results considering the effect of harbour (2 levels) and year (3 levels) on the Bayesian assignment values calculated in STRUCTURE using the complete global dataset (q1-4). See Appendix 5.3 for further details regarding the GLM output. * indicates a significant result.

Assignment cluster	Factors	df	F	*p*
q1	Harbour	1	1.3914	0.2389
	Year	2	1.8938	0.1520
	Harbour x Year	2	1.4212	0.2428
q2	Harbour	1	0.3905	0.5324
	Year	2	0.1114	0.8946
	Harbour x Year	2	5.5591	0.0042*
q3	Harbour	1	0.2408	0.6240
	Year	2	0.0089	0.9911
	Harbour x Year	2	0.1684	0.8451
q4	Harbour	1	0.4064	0.5242
	Year	2	1.3931	0.2496
	Harbour x Year	2	1.8368	0.1608

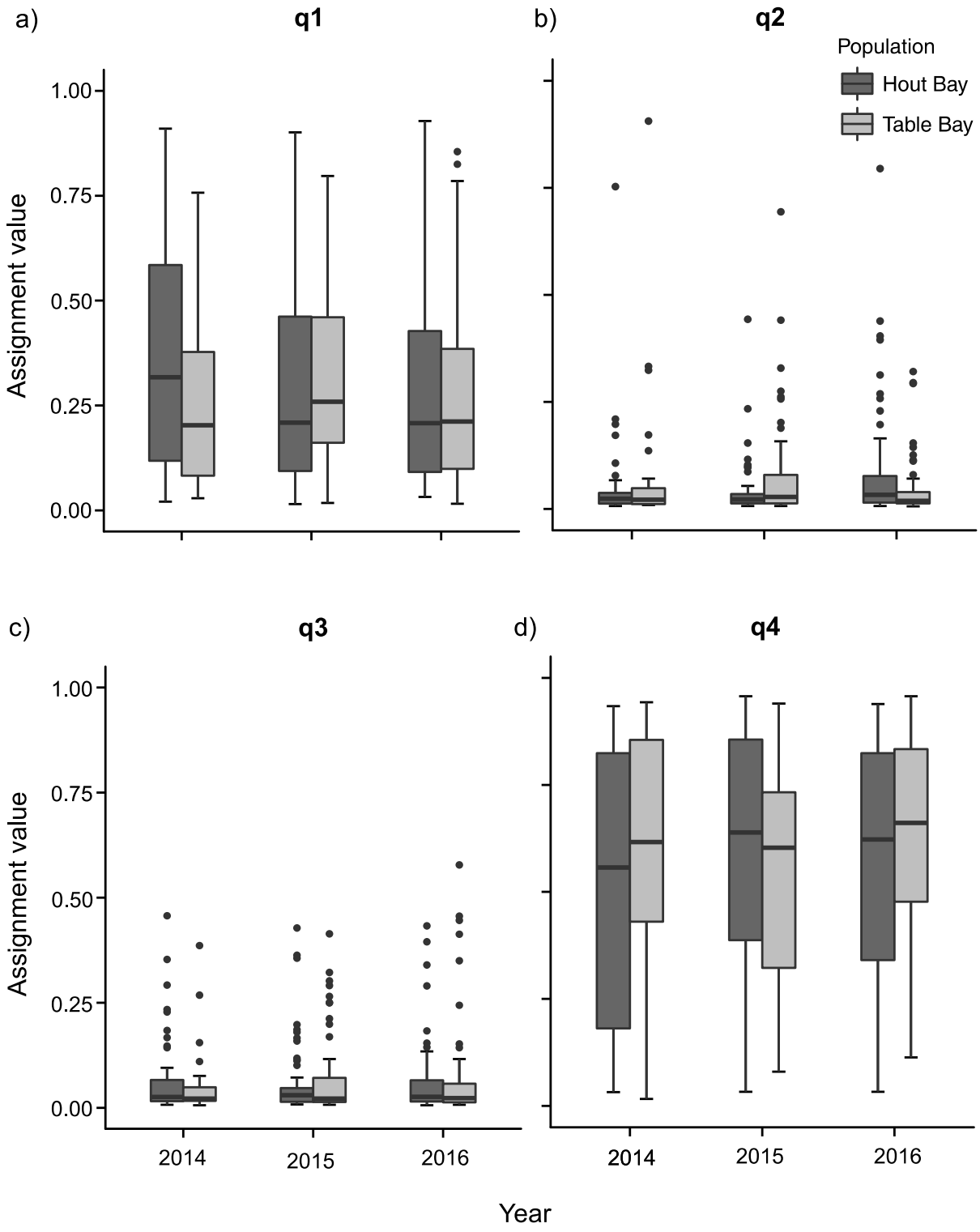

Figure 5.9. Temporal changes (2014-2016) to Bayesian assignment values in the Hout Bay and Table Bay populations with respect to four identified genetic clusters: a) q1 (predominantly Australian cluster); b) q2 (*C. aestuarii* cluster); c) q3 (North American cluster); and d) q4 (*C. maenas* native cluster).

Similarly, there was no effect of year (X^2 = 12, df = 10, p = 0.285) or harbour (X^2 = 6, df = 6, p = 0.306) on estimates of contemporary N_e. The upper 95% confidence intervals estimated for contemporary N_e were infinitesimal for all populations sampled (Table 5.10).

Table 5.10. Estimates of contemporary N_e and associated 95% jackknife confidence intervals with a P_{crit} of 0.02.

Harbour	Year	N_e	Lower 95% CI	Upper 95% CI
Hout Bay	2014	397.5	117.2	∞
	2015	483.1	170.3	∞
	2016	520.6	147.3	∞
Table Bay	2014	173.5	48.6	∞
	2015	589.7	204.5	∞
	2016	258.5	110.5	∞

Discussion

This study found that globally invasive *Carcinus* populations (excluding South Africa) showed significantly lower genetic diversity compared to populations from native regions, indicative of founder effects and low propagule pressure in these regions. These findings fit the general observation that introduced populations often exhibit reduced genetic diversity compared to their native range source populations (Dlugosch & Parker 2008).

The overall global assignment analysis found evidence for *C. aestuarii* genotypes in the native range of *C. maenas*. An oceanographic mixing zone for the Atlantic and Mediterranean waters, off the coast of Spain in the Gibraltar Strait (Tintore et al. 1988) has been proposed as the site of phylogeographic splits between Atlantic and Mediterranean populations of several marine organisms. These include the Mediterranean mussel, *Mytilus galloprovincialis* (Quesada et al. 1995) and the European flat oyster, *Ostrea edulis* (Saavreda et al. 1995). Based on the evidence for divergence of these species, it lends support to the suggestion that this zone is also the site of *Carcinus* divergence (Geller et al. 1997) and potentially represents a source population for South Africa. Gibraltar port is an important contributor to contemporary and historical global species flow within this zone as a result of its position at the entrance to the Mediterranean Sea (Xu et al. 2014).

Introductions of *Carcinus* crabs into South Africa facilitated the co-introduction of both *C. maenas* and *C. aestuarii* genotypes. STRUCTURE analyses indicated that genotypes representing possible hybrids between the two species are present in South Africa, a notion supported by previous work (Geller et al. 1997, Darling et al. 2008). The most parsimonious introduction scenario inferred through the ABC approach suggests that South African

Carcinus populations originated from an admixture event between native and globally invasive *C. maenas* populations. Given this most likely invasion scenario, the presence of *Carcinus* hybrids in South Africa might have arisen under various circumstances. The hybrids may have existed in the source population and were directly introduced to South Africa. Alternatively, one of the source populations might have been in a region where the two species are sympatric (as discussed above). Although there are no known neighbouring native and invasive populations of *C. maenas*, it is possible that larvae were collected in multiple ports by international vessels, leading to the mixing of species in the ballast water tanks, or in South Africa upon ballast discharge. If the latter scenario were true, *C. aestuarii* individuals might be detected in South Africa, whereas this study only found evidence of hybrids. The third possibility is that there were uneven introductions of the two species and many more *C. maenas* individuals were introduced than its congener. Random sampling may therefore result in low detection of pure *C. aestuarii* individuals.

In contrast to the international invaded regions included here, South African populations showed high genetic diversity, comparable to that present in native range populations. Despite the higher diversity and levels of hybridization detected in South Africa, *Carcinus* populations in Hout Bay and Table Bay harbours represent a single genetic cluster (K = 1) with very low or no population differentiation between them. Therefore, these two populations are unlikely to be the result of independent introductions and/or experience frequent gene flow. A more parsimonious explanation is that one or more secondary dispersal events occurred between these two harbours. Historical observations fit these expectations. Since the initial discovery of *Carcinus* in Table Bay harbour in 1983 (Joska & Branch 1986), the species has not spread further than 60 km either side of the initial introduction site (see Chapter 2). *Carcinus* was only discovered for the first time in Hout Bay harbour in 2002 (Robinson et al. 2005) and at several sites between the two large harbour populations. Surprisingly, the approximate Bayesian computation estimated that *Carcinus* was introduced to South Africa earlier than previously thought, i.e. in 1873 (95%CI: 1678 to 1980). However, there is a wide confidence interval encompassing that date and considering that the crab is highly conspicuous, it is unlikely to have gone undetected for so long in such a well-studied region (Awad et al. 2002). The discharge of ballast water in South African waters began in the late 1800s and completely replaced the use of dry ballast by the 1950s (Griffiths et al. 2009). However, the first detection of an alien species that was thought to have arrived in South Africa via ballast water occurred in the 1940s (Griffiths et al. 2009). Therefore, it is plausible that the upper range of introduction dates estimated by the ABC analysis, encompasses the true arrival date of the crab.

Secondary dispersal within South Africa might have occurred through natural or human-mediated transfer of propagules. It is possible that coastal advective currents flow in a southerly direction this area (Nelson & Polito 1987), enabling larval dispersal from Table Bay to Hout Bay harbour. An experimental study in this region found adult *C. maenas* are unable

to effectively grip rocky substrata under high wave action (Hampton & Griffiths 2007) which typifies much of the intertidal coastline between Hout Bay and Table Bay. As such, environmental conditions might limit the establishment of adults outside protected harbour areas (Chapter 2). Alternatively, predatory driven biotic resistance, might restrict the spread of the crabs (Chapter 4). In terms of human mediated spread, the most plausible vectors between the two harbours are pelagic fishing vessels and recreational yachts. However, in the absence of clear evidence, it is not possible to determine which vector was responsible for the initial introduction and maintenance of genetic diversity found in South African populations.

Tracking temporal changes in genetic diversity can be useful in assessing the demographic responses of invasive species to disturbances and management interventions. For example, three years after a ten-year long eradication programme against *Spartina alterniflora* in California, significant changes in genetic diversity of the species were observed (Ort & Thornton 2016). In the current study, similar and high levels of genetic diversity in *Carcinus* populations were maintained over three years in both Table Bay (unmanaged) and Hout Bay (managed) harbours. Similarly, there were no changes in N_e recorded over this time period in Hout Bay, despite the considerable reduction of the population (as modelled in Chapter 3). According to the model, the population in April 2015 was comprised of 2,360 crabs (95% CI: 937 – 4,058), while the N_e estimates from this current study indicate that the effective population size was 483 individuals (95% CI: 170 - ∞). The ratio between N_e and the census size (N_c) is approximately 0.205. This is a common trend in marine species, although usually the difference between the two estimates may be greater (Hauser & Carvalho 2008, Zeller et al. 2008, Waples & Do 2010). For example, freshwater copepod species exhibit a N_e/N_c ratio in the range of 1×10^{-7} to 1×10^{-8} (Zeller et al. 2008). However, such estimates should be considered carefully, especially for *Carcinus* in South Africa, as the crab is known to breed throughout the year and as a result has overlapping generations that might skew the ratio. There are three possible explanations for the apparent stability of the Hout Bay population, based on N_e, through time. The most likely explanation is that the diversity and structure trends simply reflects the presence of juveniles in the population who were below the minimum catchable size during the period of management but have since grown to a sufficient size to be retained in the traps. These juveniles would carry the same standing genetic variation as the previous generation. Alternatively, the diversity and structure could remain stable if management targeted all individuals equally. Therefore, although the population was considerably reduced, the same genotypes were retained within the surviving populations. Notably, this would validate the assumption of population estimates (Chapter 3). The third explanation is that there was continued migration of individuals from Table Bay during management that maintained the genetic diversity and structure of the original Hout Bay population. Although possible, this is unlikely to have occurred to such a degree that no effect of management was observed if management did indeed lead to a decrease in N_e**.** The likely explanation is that management was insufficiently severe to lower

the population size so that there was an effect on genetic diversity through strong genetic drift. Essentially, the N_e was large enough to buffer the effect of random fluctuations in genetic diversity.

In conclusion, this study has identified a plausible introduction history for *C. maenas* in South Africa, indicating an admixed origin between globally native and invasive populations of *C. maenas*. It is possible that the introduction of the crab occurred a number of years prior to its detection in 1983. The South African *Carcinus* populations are highly diverse, with similar levels of diversity and structure to the native populations. This study has confirmed the presence of *C. maenas* X *C. aestuarii* hybrids in South Africa, suggesting that at least one of the South African source populations contained hybrids or *C. aestuarii* individuals. When considering the harbours in more detail, Table Bay and Hout Bay showed no genetic structure, no changes in diversity through time, and minimal impact of management interventions on the latter. As a result, it can be concluded that the two harbour populations were unlikely to be the result of independent introductions. This provides essential information for the management of *Carcinus* in South Africa as it shows that migration is probable between the harbours, although it is unlikely to have occurred during this study period, suggesting it would be ineffective to manage a single population in isolation.

CHAPTER 6. THESIS SYNTHESIS

The genus *Carcinus* is found on five continents and is associated with notable ecological and economic impacts where it occurs as an invasive alien species (Ropes 1968, Davis et al. 1998, Grosholz et al. 2000, Lovell et al. 2007, de Riviera et al. 2011, Grosholz et al. 2011, Malyshev & Quijon 2011, Freeman et al. 2013, Mach & Chan 2013, Whitlow et al. 2010). Based on these impacts, *Carcinus* was listed in the South African national legislation as a species requiring control (NEMBA Alien and Invasive Species Lists 2016). However, previous research conducted on this genus in South Africa (Le Roux et al. 1990, Robinson et al. 2005) was outdated in terms of the assessment of the crab's distribution and abundance, while ecological impacts had not been quantified. No management has been attempted to date. Global analyses of *Carcinus* genetics have previously been undertaken (Geller et al. 1997, Darling et al. 2008), however, these studies considered only one South African population and these samples were collected over 20 years ago. Against this backdrop, this thesis aimed to conduct a comprehensive assessment of the crab's current distribution and status, trophic impacts and the historical and contemporary genetic patterns shaping its invasion in South Africa. The highlight of this study was the undertaking of the first management programme of a marine invasive species in Africa.

This thesis began with a thorough survey of the intertidal and subtidal range of *Carcinus* on the Cape peninsula and surrounding areas (Chapter 2). It included surveys of areas where the crab had previously been recorded and sites at the edge of this range from where the crab was not known but where suitable habitat was present. The intertidal and subtidal ranges previously reported along the coast had contracted and no crabs were detected in any new locations. This retraction in the crab's distribution is unique to South Africa, with all other international populations demonstrating considerable range expansions (Blakeslee et al. 2010, Koike & Iwasaki 2011, Behrens Yamada et al. 2017) with the exception of Australia (Garside et al. 2014). In South Africa *Carcinus* is primarily confined to two harbour populations, in Table Bay and Hout Bay. A demographic assessment of the Table Bay population found no evidence of a temporal reproductive peak, with gravid females found in most months and no postlarvae detected on the settlement collectors. This contrasted with international populations of *Carcinus*, where clear seasonal peaks are observed and are thought to be correlated with water temperature (Rasmussen 1973, Dries & Adelung 1982, Berrill 1982, Moksnes 1999). Such findings have implications for management of the crab in South Africa as there is no pre-reproductive window in which management action could have the greatest impact. It is recommended that monitoring of suitable crab habitat in sensitive areas such as national parks should continue as early detection can increase the probability of successful management should the crab spread to these important areas.

Given the legal status of *Carcinus* in South Africa, management of the crab was required (Chapter 3). Hout Bay harbour was selected as the site for a management trial due to it

being smaller than Table Bay and the lack of international commercial shipping traffic. The most recent population estimate (Jooste 2013) was used as a guideline for the expected duration of management. Following a trial of potential control methods, baited traps were deemed the most cost-effective in the South African context. However, these traps only targeted individuals which had a carapace width of > 35 mm, therefore, leaving a gap in the methodology whereby juveniles < 35 mm were unavoidably neglected. Once management commenced, it became clear that the previous population estimate was an underestimate and extirpation was unachievable. This inability to remove all *Carcinus* individuals reflects the outcomes of all international management programmes targeting the crab to date. A total of 36,244 crabs were removed from the harbour during intensive management action over 12 months, with a decrease in catch per unit effort (CPUE) each month. Monitoring of the population over the following six months showed a rapid recovery with an overall increase in CPUE. These findings prompted the development of a Bayesian depletion model to better understand the dynamics underlying management interventions, by drawing on techniques used in commercial fisheries with a novel application to the management of invasive species. This model predicted that, although a large proportion of the Hout Bay population was removed through management with only an estimated 984 individuals remaining, recruitment into the population continued. Given the catch patterns for large and extra-large crabs (that decreased as management continued), it is likely that the reproductive output was lowered through time. This could affect subsequent cohorts and render the population unstable. However, as small crabs might have avoided capture previously due to their size, the recruitment pattern modelled could reflect the entrapment of the next cohort of crabs. In order to achieve an 86% estimated probability of extirpation, the management effort would need to be doubled over a period of 18 months (from a mean of 3,250 to 8,000 hours of effort per month). At this level of effort, when extrapolating to the area invaded by both harbour populations, a national eradication programme is expected to cost over ZAR 60 million. This cost should be considered in a national context, where the total budget of the Department of Environmental Affairs for the management of biological invasions was ZAR 1.22 billion in 2014 (DEA 2014). Of this, ZAR 1.2 billion was secured for a public works programme (Working for water – an alien plant clearing programme) leaving 1.6% of the total budget for the management of all other invasions.

This work has highlighted the usefulness of depletion models in invasive species management. Their use should be encouraged in this field as they can provide a holistic overview of progress and evidence upon which to base management decisions. Estimates obtained in this way can assist in administrative aspects of management, by providing an indication of programme duration, as well as the level of effort and the financial resources required. Chapter 3 has paved the way for adaptive management, with many lessons learned that can help increase the probability of success of invasive species management in South Africa. Moreover, it highlighted the importance of a flexible management approach to accommodate the unexpected outcomes which often arise in field studies.

Only one study to date has addressed the impacts of *Carcinus* in South Africa (Le Roux et al. 1990). That study eluded to the impacts of the crab based on predation experiments and gut content analyses (Le Roux et al. 1990). However, neither of these methods can demonstrate actual impacts, but instead infer a potential effect. Chapter 4 addressed these shortfalls by taking advantage of the unique opportunity provided by the management intervention in Hout Bay to assess the realised trophic impacts of the crab in the field. The benthic community in Hout Bay was not significantly different after management, suggesting that the crab had no evident impact on organisms inhabiting the harbour benthos. In contrast, the fouling community changed with time in both Hout Bay and the control (Table Bay), and accordingly few conclusions can be drawn from these results. Tethering experiments were used to assess the impact of the crab on upper trophic levels. Following 126 hours of tethering in Hout Bay, no crab attacks were recorded and few predators were identified from the video footage. This could reflect the disinterest predators had in the crabs as prey, or the availability of alternate food sources, such as fisheries discards from the fish processing plants and fishing vessels in the harbour. This finding prompted aquarium experiments in which it was found that the *Carcinus* was fatally attacked by native fish, suggesting that if the crab was to expand its range beyond its current distribution, there might be some biotic resistance from native predatory fish. Here, the aquarium was used as a proxy for the natural habitat commonly found along the South African coast to give an indication of behavioural responses that may manifest in the field. However, the relative densities of the fish in the aquarium were unavoidably higher than those found in natural systems, so the speed of response and the species demonstrating interest should be considered within this context. This study highlights the context-dependent nature of invasions, whereby a species might have an impact in one region but not another. It is possible that *Carcinus* is experiencing a prolonged time lag in terms of ecological impact, where the per capita effect of the crab may increase with time (Crooks 2005). This could arise if the crab was to switch prey preferences through time, for example, reducing predation rates on invasive bivalves such as *Mytilus galloprovincialis*, in favour of the native mussel, *Aulacomya atra.* Similarly, despite the lack of evident impact currently, changes in climate could affect invasion success, and result in the future realisation of impacts of invasive species in South Africa. This could result from environmental changes which could exacerbate the impact of the crab. Current knowledge suggests that such changes are unlikely to be driven by changing water temperatures. Waters off the west coast of South Africa have been cooling over the last few decades (Rouault et al. 2010) but this trend is unlikely to affect the survival of the crab as the water temperature would remain considerably higher than the minimum temperature for survival (Behrens Yamada 2001). However, such global climate change might also alter the dynamics of invasion vectors, for example, *Carcinus* could spread into new source areas, with previously low-risk routes providing new avenues for introduction to South Africa. Such

nuances should be taken into consideration when conducting risk analyses that often incorporate the impacts recorded elsewhere into species specific risk assessments.

Molecular approaches are becoming increasingly common in the study of invasive species (Rius & Darling 2014). Chapter 5 used eight microsatellite loci to look at the historical and contemporary genetic patterns between South Africa and eight other international *Carcinus* populations. In accordance with previous studies (Geller et al. 1997, Darling et al. 2008), South Africa demonstrated high genetic diversity, similar to the native range and unlike any other invasive population sampled. Structurally, the South African populations were grouped together with the native populations of *C. maenas*, however *C. maenas* X *C. aestuarii* hybrids were detected (5% of the sampled population). Considering the absence of *C. aestuarii* individuals in South Africa, such a pattern could arise through mitochondrial introgression in the native range (where hybrids were also observed). Mitochondrial introgression has been observed in a *Carcinus* population previously, specifically the *C. aestuarii* invasion in Japan (Darling 2011). The presence of hybrids might also explain the lack of evidence of ecological impacts (Chapter 4) as interspecific hybrids might have different ecological traits than the parent species, as suggested by Darling (2011).

The most probable colonisation scenario as derived from the approximate Bayesian computations (ABC) inferred that South Africa was invaded following an admixture event between the populations in the *C. maenas* native and invasive ranges. Given the lack of neighbouring native and invasive regions, this finding might be explained by an admixture event taking place in South Africa, or the collection of propagules from multiple regions by ships en route to South Africa. The ABC analysis suggests that the arrival of *Carcinus* in South Africa might have occurred prior to its detection in 1983 (Joska & Branch 1986), though it seems unlikely that crabs went undetected for long. Prior to management, Hout Bay and Table Bay had a similar lack of structure and diversity and this did not change through time. This suggests that either the populations were subjected to high propagule pressure initially, or migration had occurred between the two populations, although no conclusions can be drawn regarding the frequency of such gene flow. The diversity, structure and effective population size of Hout Bay did not change as a result of management. Although migration from Table Bay could explain this, it is unlikely that the vector strength between the two harbours is sufficient to maintain the observed genotypes. Moreover, considering the unsuccessful targeting of juveniles during management, the next cohort of individuals might have achieved a catchable size so that they were later caught in baited traps. If this were the case, this cohort would carry the standing genetic diversity of the previous generation. Chapter 5 built on previous outdated studies, through the elucidation of the crab's South Africa invasion history, concluding that a potentially continuous propagule pressure would render management attempts futile in Hout Bay. In order to prevent future introductions, vectors should be managed. The most likely vectors responsible for the introduction of *Carcinus* are biofouling on oil rigs or commercial ships or

ballast water discharge (Mead et al. 2011b). South Africa is signatory to the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004) which was recently ratified. However, there is currently no local legislation in support of the international regulatory framework and therefore the positive effects may not be fully realised in South Africa for some time.

This study incorporated the tools from several disciplines, including ecology, genetics and environmental resource management, to improve our knowledge through the updating of previous studies and providing an important theoretical understanding of the *Carcinus* invasion. The crab's current range has contracted and evidence of year-round reproduction detected. The management trial was the first of its kind in Africa and highlighted several administrative and scientific insights. This included the novel application of depletion models to invasive species management. Trophic impacts on the harbour communities were not evident, in contrast to other ranges where ecological impacts have been recorded. The crab's inferred invasion history has answered some important questions regarding the source populations and the year of introduction. Overall, this comprehensive study has furthered our understanding of *Carcinus* in South Africa.

In conclusion, considering the crab's limited distribution, the lack of evident ecological impacts on harbour communities, the continued potential for re-introduction, the unavailability of secure financial resources and the inability of management to target all life stages, a national eradication programme is not recommended. The only management action proposed is to monitor populations. If crabs were to invade other parts of South Africa and particularly if they could adapt to natural environments, the management approach would need to be reassessed, and either an extirpation attempt made, or some long-term maintenance management implemented (e.g. biological control). Better information on introduction dynamics would assist in choosing between these options. While there is little evidence that *Carcinus* invasions in South Africa have transformed natural habitat, the impacts seen elsewhere are probably sufficient to validate the retention of this crab as a listed alien species under the NEM:BA Alien and Invasive Species Regulations.

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APPENDICES

Appendix 2.1.

Total monthly catches and mean catch per unit effort (CPUE) for crabs with a carapace width of ≤ 48 mm to allow a comparison between baited traps and crab condos in Table Bay harbour.

Month	Baited trap catch	Condo catch	Baited CPUE	Condo CPUE
March '14	13	0	1.63	0.00
April '14	19	0	2.38	0.00
May '14	55	7	6.88	0.07
June '14	93	4	11.63	0.04
July '14	51	2	6.38	0.02
August '14	55	2	6.88	0.02
September '14	14	2	1.75	0.02
October '14	50	0	6.25	0.00
November '14	31	2	3.88	0.02
December '14	12	1	1.50	0.01
January '15	19	1	2.38	0.01
February '15	0	0	0.00	0.00

Appendix 3.1.

Bycatch collected in the baited traps during management of *Carcinus* in Hout Bay harbour, with the number of individuals belonging to each taxon.

Taxa	Common name	Total number caught
CHORDATA		
Chondrichthyes		
***Haploblepharus* sp.**	Shy shark	2,274
Osteichthyes		
Clinus sp.	Klipfish	27,695
Aves		
***Phalacrocorax* sp.**	Cormorant	5
MOLLUSCA		
Gastropoda		
***Pleurobranchaea* sp.**	Sea slug	1,625
ARTHROPODA		
Decapoda		
Cyclograpsus punctatus	Shore crab	5
Jasus lalandii	West coast rock lobster	603
Ovalipes trimaculatus	Three-spot swimming crab	48
Plagusia chabrus	Cape rock crab	462
Hymenosoma orbiculare	Crown crab	279
ECHINODERMATA		
Echinoidea		
Parechinus angulosus	Cape urchin	96

Appendix 3.2.

GLM results considering the effect of crab size category (4 levels), sex (2 levels) and month (12 levels) on catch per unit effort (CPUE) during active management.

Factor	df	Deviance	Residual df	Residual deviance	F	*p*
Month	12	65,682	165,435	364,557	1,538.97	<0.0001
Size	3	93,310	165,432	271,247	8,745.21	<0.0001
Sex	1	34,620	165,431	236,627	9,733.89	<0.0001
Month x Size	36	8,639	165,395	227,988	67.47	<0.0001
Month x Sex	12	4,935	165,383	223,053	115.62	<0.0001
Size x Sex	3	1,441	165,380	221,612	135.07	<0.0001
Month x Size x Sex	36	355	165,344	221,257	2.77	<0.0001

Appendix 3.3.

GLM results of catch per unit effort (CPUE) during the monitoring phase with respect to the main effects of crab size category (4 levels), sex (2 levels) and month (6 levels).

Factor	df	Deviance	Residual df	Residual deviance	F	*p*
Month	5	269.25	1,914	3,247.1	20.45	<0.0001
Size	3	643.40	1,911	2,603.7	81.46	<0.0001
Sex	1	30.17	1,910	2,573.5	11.46	0.0007
Month x Size	15	137.85	1,895	2,435.6	3.49	<0.0001
Month x Sex	5	50.60	1,890	2,385.0	3.84	0.0018
Size x Sex	3	94.97	1,887	2,290.1	12.02	<0.0001
Month x Size x Sex	15	30.00	1,872	2,260.1	0.76	0.7237

Appendix 4.1.

PERMANOVA (main test results) from the benthic communities collected in Table Bay and Hout Bay harbours in 2014, 2015 and 2016.

Factor	Groups	df	SS	MS	Pseudo-F	*p*
Harbour	Hout Bay, Table Bay	1	15,270	15,270	6.34	0.001
Year	2014, 2015, 2016	2	14,771	7,385.5	3.07	0.001
Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	2	13,523	6,761.3	2.81	0.001

Appendix 4.2.

PERMANOVA (pairwise test results) from the benthic communities collected in Table Bay and Hout Bay harbours in 2014, 2015 and 2016.

Factor	Groups	Denominator df	t	*p*
Harbour x Year: 2014	Hout Bay, Table Bay	7	1.37	0.090
Harbour x Year: 2015	Hout Bay, Table Bay	7	2.15	0.009
Harbour x Year: 2016	Hout Bay, Table Bay	7	2.69	0.011
Harbour x Year: Hout Bay	2014, 2015	6	0.56	0.899
	2014, 2016	6	0.92	0.589
	2015, 2016	6	1.06	0.364
Harbour x Year: Table Bay	2014, 2015	8	2.57	0.008
	2014, 2016	8	2.21	0.009
	2015, 2016	8	4.10	0.008

Appendix 4.3.

PERMANOVA (main test results) from the fouling communities collected in Table Bay and Hout Bay harbours in 2014, 2015 and 2016.

Factor	Groups	df	SS	MS	Pseudo-F	*p*
Harbour	Hout Bay, Table Bay	1	17,902	17,902	16.15	0.001
Year	2014, 2015, 2016	2	20,432	10,216	9.22	0.001
Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	2	7,834	3,917	3.53	0.001

Appendix 4.4.

PERMANOVA (pairwise test results) from the fouling communities collected in Table Bay and Hout Bay harbours in 2014, 2015 and 2016.

Factor	Groups	Denominator df	t	*p*
Harbour x Year: 2014	Hout Bay, Table Bay	5	2.82	0.045
Harbour x Year: 2015	Hout Bay, Table Bay	7	2.67	0.004
Harbour x Year: 2016	Hout Bay, Table Bay	8	3.19	0.005
Harbour x Year: Hout Bay	2014, 2015	4	1.19	0.262
	2014, 2016	5	2.54	0.036
	2015, 2016	7	3.05	0.008
Harbour x Year: Table Bay	2014, 2015	8	3.24	0.007
	2014, 2016	8	3.16	0.008
	2015, 2016	8	2.09	0.012

Appendix 4.5.

GLM results of benthic community diversity with respect to site (two levels) and year (three levels).

Factor	Groups	df	Deviance	Residual df	Residual deviance	F	*p*
Harbour	Hout Bay, Table Bay	1	2.29	24	9.16	8.25	0.0086
Year	2014, 2015, 2016	1	0.27	25	11.45	1.70	0.2046
Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	1	0.28	23	8.85	0.10	0.3279

Appendix 4.6.

GLM results of fouling community diversity with respect to site (two levels) and year (three levels).

Factor	Groups	df	Deviance	Residual df	Residual deviance	F	*p*
Harbour	Hout Bay, Table Bay	1	0.10	23	2.96	1.23	0.2794
Year	2014, 2015, 2016	1	0.06	24	3.06	0.74	0.3981
Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	1	1.05	22	1.92	13.13	0.0015

Appendix 4.7.

GLM results of the number of interested species with respect to sex (two levels) and size class (three levels).

Factor	Groups	df	Deviance	Residual df	Residual deviance	F	*p*
Sex	Male, Female	1	0.06	32	18.62	0.12	0.7286
Size class	Small, Medium, Large	2	5.57	33	18.69	5.29	0.0108
Sex x Size class	Small male, Medium male, Large male, Small female, Medium female, Large female	2	2.22	30	16.40	2.11	0.1388

Appendix 4.8.

GLM results of a) the time to the first attack with respect to sex (two levels) and size class (three levels) and b) the time to the fatal attack with respect to sex (two levels) and size class (three levels).

Factor	Groups	df	Deviance	Residual df	Residual deviance	F	*p*
a) *Time to first attack*							
Sex	Male, Female	1	1.18	34	31.02	1.23	0.2764
Size class	Small, Medium, Large	2	2.12	32	28.90	1.10	0.3451
Sex x Size class	Small male, Medium male, Large male, Small female, Medium female, Large female	2	4.92	30	24.88	2.10	0.1405
b) *Time to fatal attack*							
Sex	Male, Female	1	0.06	34	34.73	0.05	0.8266
Size class	Small, Medium, Large	2	2.68	32	32.06	1.16	0.3271
Sex x Size class	Small male, Medium male, Large male, Small female, Medium female, Large female	2	4.06	30	27.10	1.76	0.1896

Appendix 4.9.

GLM results of the number of attacks with respect to sex (two levels) and size class (three levels).

Factor	Groups	df	Deviance	Residual df	Residual deviance	F	*p*
Sex	Male, Female	1	10.52	34	190.31	2.86	0.1010
Size class	Small, Medium, Large	2	63.47	32	126.85	8.64	0.0011
Sex x Size class	Small male, Medium male, Large male, Small female, Medium female, Large female	2	15.60	30	111.25	2.12	0.1373

Appendix 5.1.

Details of the 23 trialled microsatellite loci.

Original study	Original locus name	Sequence	Repeat sequence	Anneal temp.	Size range
Marino et al. 2008	**Cae01**	F: CCTAACCTATCTCAACAGTG R: CTGCTTGCCTTGTTCGTA	$(CAT)_{12}CGT(CAT)_8$	53	144-188
Marino et al. 2008	**Cae07**	F: TGGCTCTTTTCACCTTCTGT R: CTATCGGAAGAGACGCAAGA	$(GA)_{28}$	50	223-367
Marino et al. 2008	**Cae14**	F: AACACATCAGTGAGTGCCAC R: CTAAGCCATCTGGAATCACG	$(CACAGA)_9CAGA\ (CACAGA)_2GAGA\ (CACAGA)$	57	197–239
Marino et al. 2008	**Cae17**	F: ACTCACCTCCGTCATCACC R: TCCTGAGCTCCATCAAGACT	$(CA)_4GC(CA)_{13}\ GC(CA)$	55	145–165
Marino et al. 2008	**Cae30**	F: TACAGGGTAAGCCACAGTCC R: GCAGAAGAGGTCACGAGAAG	$(GT)_9$	55	243–249
Marino et al. 2008	**Cae33**	F: CCATACAGGGCTGTACAGAAA R: CGGAATCTCGGTTATATGGCTT	$(AC)_{13}TTTT(AC)_9$	52	267–335
Marino et al. 2008	**Cae59†**	F: TGTGGAGACAGGACACAATG R: ATAAGGAGTCGTAACAGGGA	$(CA)_{22}$	51	220–264
Marino et al. 2008	**Cae71**	F: CCAGGACTAACAGTGGGTGA R: TGCATGTTAGCCACTCCTCT	$(GT)_{17}$	56	200–276
Marino et al. 2008	**Cae86**	F: GCGGTGAAGAAGATGGAGTA R: GCGAGCTCAAATGAATAATGGT	$(GA)_{22}$	51	198–236
Marino et al. 2008	**Cma02EPA**	F: TTGATCCATATATCTATTCTTTCTTGC R: TTTGGGGGAAATACTTATCTCTT	$(TAGA)_7$	52	208–314
Marino et al. 2008	**Cma04EPA**	F: GAGCTCCAGGAAACTGTATCTGA R: GCCCTCTATCTCGCTTTATATCTC	$(TAGA)_{10}$	56	127-201

Tepolt et al. 2006	**Cma03EPA**	F-CGCTCGACATGCTGTATTGT R-CAATTTATCTATCCATCTCTATCCTTC	$(TAGA)_{16}$	55	157–185
Tepolt et al. 2006	**Cma06EPA**	F-TCGCTTCGTATTCACGTCCT R-CCCACCCTAAGGTAACACGA	$(TAA)_{1}$	66	241-244
Tepolt et al. 2006	**Cma07EPA**	F-TCAGGGCCAAAAGTTATTCAA R-GTTGTTGGCATTCGCTCTTT	$(TAA)_{21}$	57	156-180
Tepolt et al. 2006	**Cma08EPA**	F-TCAAGGTCAGAGAGGAGGATG R-GTGAGGCGAGCGTGCTAAC	$(TC)_{18}$	55	129–157
Tepolt et al. 2006	**Cma09EPA**	F-CAAAACACAAAGCAAAATGGTC R-CCTTGCTCGAACCCTGTAAA	$(GT)_{13}$	65	178-184
Tepolt et al. 2006	**Cma10EPA**	F-GAGACCGTCAATGCAGCTTCCTCT R-GGGACAGAACGTATCTAGGTCACC	$(GT)_{37}$	66	249–289
Tepolt et al. 2006	**Cma12EPA**	F-TGCAACACAGCAACACAAGA R-GTGGTAGGATGCGGCAAAG	$(GT)_{38}$	62	160–186
Tepolt et al. 2006	**Cma13EPA**	F-CTATCACGAAGGGATGGTGAG R-CCAGGGAAAGATAAGGGTAAGG	$(GT)_{41}$	62	165–189
Tepolt et al. 2006	**Cma14EPA**	F-ACGGCTCACCTACGTGCACT R-GGCTGTGGTCCTGTGTTCATT	$(GGT)_{8}$	62	256–259
Pascoal et al. 2009	**SP107**	F:GTACCCGGGAAGCAGAGAAC R:CACTTGCTATAAAGGCCTCAGC	$(GAG)_{16}$	51	150-189
Pascoal et al. 2009	**SP251**	F:TGGTACTGTGCGTGGTGAAGC R:TGTGGTACGATGCGGCATAG	$(CA)_{38}$	59	201-269
Pascoal et al. 2009	**SP495**	F:AAGTTCCAGGGCCTGAGTGTA R:TAGTGGTGGTGGTGGTGGAAT	$(CAG)_{10}$	52	142-193

Appendix 5.2.

GLM results considering the effect of harbour (2 levels and year (3 levels) on A_R.

Factor	Groups	df	Deviance	Residual df	Residual deviance	F	*p*
Harbour	Hout Bay, Table Bay	1	0.07	47	133.65	0.03	0.8663
Year	2014, 2015, 2016	2	1.06	48	113.72	0.22	0.8064
Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	2	0.16	45	133.49	0.03	0.9677

Appendix 5.3.

GLM results considering the effect of harbour (2 levels) and year (3 levels) on the Bayesian assignment values calculated in STRUCTURE using the complete global dataset (q1-4).

Assignment cluster	Factor	Groups	df	Deviance	Residual df	Residual deviance	F	*p*
q1	**Harbour**	Hout Bay, Table Bay	1	0.27	366	68.70	1.39	0.2389
	Year	2014, 2015, 2016	2	0.72	367	68.97	1.89	0.1520
	Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	2	0.54	364	68.16	1.42	0.2428
q2	**Harbour**	Hout Bay, Table Bay	1	0.07	366	35.31	0.39	0.5324
	Year	2014, 2015, 2016	2	0.04	367	35.38	0.11	0.8946
	Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	2	1.94	364	33.38	5.56	0.0042
q3	**Harbour**	Hout Bay, Table Bay	1	0.03	366	31.16	0.24	0.6240
	Year	2014, 2015, 2016	2	0.00	367	31.19	0.01	0.9911
	Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	2	0.05	364	31.11	0.17	0.8451
q4	**Harbour**	Hout Bay, Table Bay	1	0.05	366	56.76	0.41	0.5242
	Year	2014, 2015, 2016	2	0.36	367	56.81	1.39	0.2496
	Harbour x Year	Hout Bay 2014, Hout Bay 2015, Hout Bay 2016, Table Bay 2014, Table Bay 2015, Table Bay 2016	2	0.48	364	56.28	1.84	0.1608